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ERRATUM

THE BIOLOGICAL BULLETIN, Volume **160,** Number 1, Page 118

The following correction should be made in the paper by T. Pavan Kumar *et al.* entitled, Circadian fluctuations in total protein and carbohydrate content in the slug *Laevicaulis alte* (Ferussac, 1821): In Table I, the first lines of the rightmost column should read as:

−346° *

(−319.5° to −348.5°)

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III. CERTIFICATE OF ORGANIZATION

(On File in the Office of the Secretary of the Commonwealth)

No. 3170

We, Alpheus Hyatt, President, William Stanford Stevens, Treasurer, and William T. Sedgwick, Edward G. Gardiner, Susan Mims and Charles Sedgwick Minot being a majority of the Trustees of the Marine Biological Laboratory in compliance with the requirements of the fourth section of chapter one hundred and fifteen of the Public Statutes do hereby certify that the following is a true copy of the agreement of association to constitute said Corporation, with the names of the subscribers thereto:-

We, whose names are hereto subscribed, do, by this agreement, associate ourselves with the intention to constitute a Corporation according to the provisions of the one hundred and fifteenth chapter of the Public Statutes of the Commonwealth of Massachusetts, and the Acts in amendment thereof and in addition thereto.

The name by which the Corporation shall be known is THE MARINE BIOLOGICAL LABORATORY.

The purpose for which the Corporation is constituted is to establish and maintain a laboratory or station for scientific study and investigations, and a school for instruction in biology and natural history.

The place within which the Corporation is established or located is the city of Boston within said Commonwealth.

The amount of its capital stock is none.

In Witness Whereof, we have hereunto set our hands, this twenty seventh day of February in the year eighteen hundred and eighty-eight, Alpheus Hyatt, Samuel Mills, William T. Sedgwick, Edward G. Gardiner, Charles Sedgwick Minot, William G. Farlow, William Stanford Stevens, Anna D. Phillips, Susan Mims, B. H. Van Vleck.

That the first meeting of the subscribers to said agreement was held on the thirteenth day of March in the year eighteen hundred and eighty-eight.

In Witness Whereof, we have hereunto signed our names, this thirteenth day of March in the year eighteen hundred and eighty-eight, Alpheus Hyatt, President, William Stanford Stevens, Treasurer, Edward G. Gardiner, William T. Sedgwick, Susan Mims, Charles Sedgwick Minot.

(Approved on March 20, 1888 as follows:

I hereby certify that it appears upon an examination of the within written certificate and the records of the corporation duly submitted to my inspection, that the requirements of sections one, two and three of chapter one hundred and fifteen, and sections eighteen, twenty and twenty-one of chapter one hundred and six, of the Public Statutes, have been compiled with and I hereby approve said certificate this twentieth day of March A.D. eighteen hundred and eighty-eight.

CHARLES ENDICOTT
Commissioner of Corporations)

IV. ARTICLES OF AMENDMENT

(On File in the Office of the Secretary of the Commonwealth)

We, James D. Ebert, President, and David Shepro, Clerk of the Marine Biological Laboratory, located at Woods Hole, Massachusetts 02543, do hereby certify that the following amendment to the Articles of Organization of the Corporation was duly adopted at a meeting held on August 15, 1975, as adjourned to August 29, 1975, by vote of 444 members, being at least two-thirds of its members legally qualified to vote in the meetings of the corporation:

VOTED: That the Certificate of Organization of this corporation be and it hereby is amended by the addition of the following provisions:

> "No Officer, Trustee or Corporate Member of the corporation shall be personally liable for the payment or satisfaction of any obligation or liabilities incurred as a result of, or otherwise in connection with, any commitments, agreements, activities or affairs of the corporation.

> "Except as otherwise specifically provided by the Bylaws of the corporation, meetings of the Corporate Members of the corporation may be held anywhere in the United States.
>
> "The Trustees of the corporation may make, amend or repeal the Bylaws of the corporation in whole or in part, except with respect to any provisions thereof which shall by law, this Certificate or the Bylaws of the corporation, require action by the Corporate Members."

The foregoing amendment will become effective when these articles of amendment are filed in accordance with Chapter 180, Section 7 of the General Laws unless these articles specify, in accordance with the vote adopting the amendment, a later effective date not more than thirty days after such filing, in which event the amendment will become effective on such later date.

In Witness whereof and Under the Penalties of Perjury, we have hereto signed our names this 2nd day of September, in the year 1975, James D. Ebert, President; David Shepro, Clerk.

(Approved on October 24, 1975, as follows:

I hereby approve the within articles of amendment and, the filing fee in the amount of $10 having been paid, said articles are deemed to have been filed with me this 24th day of October, 1975.

PAUL GUZZI
Secretary of the Commonwealth)

V. BYLAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

(Revised August 11, 1978)

I. (A) The name of the Corporation shall be The Marine Biological Laboratory. The Corporation's purpose shall be to establish and maintain a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history.

(B) Marine Biological Laboratory admits students without regard to race, color, sex, national and ethnic origin to all the rights, privileges, programs and activities generally accorded or made available to students in its courses. It does not discriminate on the basis of race, color, sex, national and ethnic origin in employment, administration of its educational policies, admissions policies, scholarship and other programs.

II. (A) The members of the Corporation ("Members") shall consist of persons elected by the Board of Trustees, upon such terms and conditions and in accordance with such procedures, not inconsistent with law or these Bylaws, as may be determined by said Board of Trustees. Except as provided below, any Member may vote at any meeting, either in person or by proxy executed no more than six months prior to the date of such meeting. Members shall serve until their death or resignation unless earlier removed, with or without cause, by the affirmative vote of two-thirds of the Trustees then in office. Any member who has attained the age of seventy years or has retired from his home institution shall automatically be designated a Life Member provided he signifies his wish to retain his membership. Life Members shall not have the right to vote and shall not be assessed for dues.

(B) The Associates of the Marine Biological Laboratory shall be an unincorporated group of persons (including associations and corporations) interested in the Laboratory and shall be organized and operated under the general supervision and authority of the Trustees.

III. The officers of the Corporation shall consist of a Chairman of the Board of Trustees, President, Director, Treasurer and Clerk, elected or appointed by the Trustees as set forth in Article IX.

IV. The Annual Meeting of the Members shall be held on the Friday following the Second Tuesday in August in each year at the Laboratory in Woods Hole, Massachusetts, at 9:30 a.m. Subject to the provisions of Article VIII(2), at such meeting the Members shall choose by ballot six Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the Members may be called by the Chairman or Trustees to be held at such time and place as may be designated.

V. Twenty five Members shall constitute a quorum at any meeting. Except as otherwise required by law or these Bylaws, the affirmative vote of a majority of the Members voting in person or by proxy at a meeting attended by a quorum (present in person or by proxy) shall constitute action on behalf of the Members.

VI. (A) Inasmuch as the time and place of the Annual Meeting of Members are fixed by these Bylaws, no notice of the Annual Meeting need be given. Notice of any special meeting of Members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least 15 days before such meeting, to each Member at his or her address as shown on the records of the Corporation.

(B) Any meeting of the Members may be adjourned to any other time and place by the vote of a majority of those Members present or represented at the meeting, whether or not such Members constitute a quorum. It shall not be necessary to notify any Member of any adjournment.

VII. The Annual Meeting of the Trustees shall be held promptly after the Annual Meeting of the Corporation at the Laboratory in Woods Hole, Massachusetts. Special meetings of the Trustees shall be called by the Chairman, the President, or by any seven Trustees, to be held at such time and place as may be designated. Notice of Trustees' meetings may be given orally, by telephone, telegraph or in writing; and notice given in time to enable the Trustees to attend, or in any case notice sent by mail or telegraph to a Trustee's usual or last known place or residence, at least one week before the meeting shall be sufficient. Notice of a meeting need not be given to any Trustee if a written waiver of notice, executed by him before or after the meeting is filed with the records of the meeting, or if he shall attend the meeting without protesting prior thereto or at its commencement the lack of notice to him.

VIII. (A) There shall be four groups of Trustees:

(1) Trustees (the "Corporate Trustees") elected by the Members according to such procedures, not inconsistent with these Bylaws, as the Trustees shall have determined. Except as provided below, such Trustees shall be divided into four classes of six, one class to be elected each year to serve for a term of four years. Such classes shall be designated by the year of expiration of their respective terms.

(2) Trustees ("Board Trustees") elected by the Trustees then in office according to such procedures, not inconsistent with these Bylaws, as the Trustees shall have determined. Except as provided below, such Board Trustees shall be divided into four classes of three, one class to be elected each year to serve for a term of four years. Such classes shall be designated by the year of expiration of their respective terms. It is comtemplated that, unless otherwise determined by the Trustees for good reason, Board Trustees shall be individuals who have not been considered for election as Corporate Trustees.

(3) Trustees ex officio, who shall be the Chairman, the President, the Director, the Treasurer, and the Clerk.

(4) Trustees emeriti who shall include any Member who has attained the age of seventy years (or the age of sixty five and has retired from his home institution) and who has served a full elected term as a regular Trustee, provided he signifies his wish to serve the Laboratory in that capacity. Any Trustee who qualifies for emeritus status shall continue to serve as a regular Trustee until the next Annual Meeting whereupon his office as regular Trustee shall become vacant and be filled by election by the Members or by the Board, as the case may be. The Trustees ex officio and emeriti shall have all the rights of the Trustees, except that Trustees emeriti shall not have the right to vote.

(B) The aggregate number of Corporate Trustees and Board Trustees elected in any year (excluding Trustees elected to fill vacancies which do not result from expiration of a term) shall not exceed nine. The number of Board Trustees so elected shall not exceed three and unless otherwise determined by vote of the Trustees, the number of Corporate Trustees so elected shall not exceed six.

(C) The Trustees and Officers shall hold their respective offices until their successors are chosen in their stead.

(D) Any Trustee may be removed from office at any time with or without cause, by vote of a majority of the Members entitled to vote in the election of Trustees; or for cause, by vote of two-thirds of the Trustees then in office. A Trustee may be removed for cause only if notice of such action shall have been given to all of the Trustees or Members entitled to vote, as the case may be, prior to the meeting at which such action is to be taken and if the Trustee so to be removed shall have been given reasonable notice and opportunity to be heard before the body proposing to remove him.

(E) Any vacancy in the number of Corporate Trustees, however arising, may be filled by the Trustees then in office unless and until filled by the Members at the next Annual Meeting. Any vacancy in the number of Board Trustees may be filled by the Trustees.

(F) A Corporate Trustee or a Board Trustee who has served an initial term of at least 2 years duration shall be eligible for re-election to a second term, but shall be ineligible for re-election to any subsequent term until two years have elapsed after he last served as Trustee.

IX. (A) The Trustees shall have the control and management of the affairs of the Corporation. They shall elect a Chairman of the Board of Trustees who shall be elected annually and shall serve until his successor is selected and qualified and who shall also preside at meetings of the Corporation. They shall elect a President of the Corporation who shall also be the Vice Chairman of the Board of Trustees and Vice Chairman of meetings of the Corporation, and who shall be elected annually and shall serve until his successor is selected and qualified. They shall annually elect a Treasurer who shall serve until his successor is selected and qualified. They shall elect a Clerk (a resident of Massachusetts) who shall serve for a term of 4 years. Eligibility for re-election shall be in accordance with the content of Article VIII (F) as applied to Corporate or Board Trustees. They shall elect Board Trustees as described in Article VIII (B). They shall appoint a Director of the Laboratory for a term not to exceed five years, provided the term shall not exceed one year if the candidate has attained the age of 65 years prior to the date of the appointment. They may choose such other officers and agents as they may think best. They may fix the compensation and define the duties of all the officers and agents of the Corporation and may remove them at any time. They may fill vacancies occurring in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number as provided in Article X, and to delegate to such Committee such of their own powers as they may deem expedient in addition to those powers conferred by Article X. They shall from time to time elect Members to the Corporation upon such terms and conditions as they shall have determined, not inconsistent with law or these Bylaws.

(B) The Board of Trustees shall also have the power, by vote of a majority of the Trustees then in Office, to elect an Investment Committee and any other committee and, by like vote, to delegate thereto some or all of their powers except those which by law, the Articles of Organization or these Bylaws they are prohibited from delegating. The members of any such committee shall have such tenure and duties as the Trustees shall determine; provided that the Investment Committee, which shall oversee the management of the Corporation's endowment funds and marketable securities, shall include the Chairman of the Board of Trustees, the Treasurer of the Corporation, and the Chairman of the Corporation's Budget Committee, as *ex officio* members, together with such Trustees as may be required for not less than two-thirds of the Investment Committee to consist of Trustees. Except as otherwise provided by these Bylaws or determined by the Trustees, any such committee may make rules for the conduct of its business; but, unless otherwise provided by the Trustees or in such rules, its business shall be conducted as nearly as possible in the same manner as is provided by these Bylaws for the Trustees.

X. (A) The Executive Committee is hereby designated to consist of not more than ten members, including the ex officio Members (Chairman of the Board of Trustees, President, Director and Treasurer); and six additional Trustees, two of whom shall be elected by the Board of Trustees each year, to serve for a three-year term.

(B) The Chairman of the Board of Trustees shall act as Chairman of the Executive Committee, and the President as Vice Chairman. A majority of the members of the Executive Committee shall constitute a quorum and the affirmative vote of a majority of those voting at any meeting at which a quorum is present shall constitute action on behalf of the Executive Committee. The Executive Committee shall meet at such times and places and upon such notice and appoint such sub-committees as the Committee shall determine.

(C) The Executive Committee shall have and may exercise all the powers of the Board during the intervals between meetings of the Board of Trustees except those powers specifically withheld from time to time by vote of the Board or by law. The Executive Committee may also appoint such committees, including persons who are not Trustees, as it may from time to time approve to make recommendations with respect to matters to be acted upon by the Executive Committee or the Board of Trustees.

(D) The Executive Committee shall keep appropriate minutes of its meetings and its action shall be reported to the Board of Trustees.

(E) The elected Members of the Executive Committee shall constitute as a standing "Committee for the Nomination of Officers," responsible for making nominations, at each Annual Meeting of the Corporation, and of the Board of Trustees, for candidates to fill each office as the respective terms of office expire (Chairman of the Board, President, Director, Treasurer, and Clerk).

XI. A majority of the Trustees, the Executive Committee, or any other committee elected by the Trustees shall constitute a quorum; and a lesser number than a quorum may adjourn any meeting from time to time without further notice. At any meeting of the Trustees, the Executive Committee, or any other committee elected by the Trustees, the vote of a majority of those present, or such different vote as may be specified by law, the Articles of Organization or these Bylaws, shall be sufficient to take any action.

XII. Any action required or permitted to be taken at any meeting of the Trustees, the Executive Committee or any other committee elected by the Trustees as referred to under Article IX may be taken without a meeting if all of the Trustees or members of such committee, as the case may be, consent to the action in writing and such written consents are filed with the records of meetings. The Trustees or members of the Executive Committee or any other committee appointed by the Trustees may also participate in meeting by means of conference telephone, or otherwise take action in such a manner as may from time to time be permitted by law.

XIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees then in office.

XIV. These Bylaws may be amended by the affirmative vote of the Members at any meeting, provided that notice of the substance of the proposed amendment is stated in the notice of such meeting. As authorized by the Articles of Organization, the Trustees, by a majority of their number then in office, may also make, amend, or repeal these Bylaws, in whole or in part, except with respect to (a) the provisions of these Bylaws governing (i) the removal of Trustees and (ii) the amendment of these Bylaws and (b) any provisions of these Bylaws which by law, the Articles of Organization or these Bylaws, requires action by the Members.

No later than the time of giving notice of the meeting of Members next following the making, amending or repealing by the Trustees of any Bylaw, notice thereof stating the substance of such change shall be given to all Corporation Members entitled to vote on amending the Bylaws.

Any Bylaw adopted by the Trustees may be amended or repealed by the Members entitled to vote on amending the Bylaws.

XV. The account of the Treasurer shall be audited annually by a certified public accountant.

XVI. The Corporation will indemnify every person who is or was a trustee, officer or employee of the Corporation or a person who provides services without compensation to an Employee Benefit Plan maintained by the Corporation, for any liability (including reasonable costs of defense and settlement) arising by reason of any act or omission affecting an Employee Benefit Plan maintained by the Corporation or affecting the participants or beneficiaries of such Plan, including without limitation any damages, civil penalty or excise tax imposed pursuant to the Employee Retirement Income Security Act of 1974; provided, (1) that the Act or omission shall have occurred in the course of the person's service as trustee or officer of the Corporation or within the scope of the employment of an employee of the Corporation or in connection with a service provided without compensation to an Employee Benefit Plan maintained by the Corporation, (2) that the Act or omission be in good faith as determined by the Corporation (whose determination made in good faith and not arbitrarily or capriciously shall be conclusive), and (3) that the Corporation's obligation hereunder shall be offset to the extent of any otherwise applicable insurance coverage, under a policy maintained by the Corporation or any other person, or other source of indemnification.

VI. REPORT OF THE DIRECTOR

Introduction

I will depart somewhat, in this year's report, from the organization used for it in the recent past—a summary of Laboratory activities under such heads as *Research, Education, Finances, Management.* This is not because information so reduced and assembled is not of vital interest to the Corporation, but rather because such information is now available in other reports and publications, a number of which result from our increased effort of public communications. The pages available for this Director's Report being for practical reasons limited, it seems to me worthwhile to use this opportunity to emphasize, more directly than heretofore, specific areas of MBL operations, *i.e.*, the day-to-day work of its various Departments. My hope is, thereby, better to inform Corporation members, who may not recently have been in residence, of current responsibilities and achievements of our support staff.

In this section of the report I shall not mention staff names, there being the danger that not enough can be said about some who, like Queen Elizabeth[1], desire only a line or two. Nevertheless it can be said, with no exaggeration, that without

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[1] ". . . I am no lover of Pompous Title, but only desire that my Name may be recorded in a line or two, which shall briefly express my Name, my Virginity, the Years of my Reign, the Reformation of Religion under it, and my Preservation of Peace . . ."

Queen Elizabeth (1533–1603),
to her Ladies-in-Waiting

the unusual skills, efficiency, and loyalty of that staff, the Laboratory's research and educational product could not have continued over the transition years now behind us to be so large and of so uniquely high a quality.

Of course, the Corporation *is* kept apprised of routine operations, in a regular way, by the reports of its Standing Committees. To those committees no sufficient praise can be given. They are a body of volunteers (albeit also of owners) whose gift of labor and of time—usually taken from an already too-short period of research or teaching—is both remarkable and indispensable.

An effort, begun two years ago, to systematize membership rotation on those standing committees, seems to have met with general acceptance and to have lightened the load for some of our more generous (or more easily persuaded) colleagues. That effort will be the subject of a report to the Corporation, with recommendations, in the near future.

Financial operations and control

Financial control and management of the Laboratory has been done, almost throughout its history, by a far smaller corps than would have been assigned to it in any other organization of like importance and size. Even taking into account the invaluable contributions of Standing Committees, of the Treasurer, and of the Executive Committee, those devoted professionally to the task of piloting the organization through fog and shoal of the national economy, and of public and private support for science, have been a skeleton crew.

I choose the nautical figure of speech, with its slightly Gothic close, as a kind of introduction to the start of the current Treasurer's Report , which is to be found elsewhere in this volume. That report is far from Gothic. On the contrary, it is happy information, signaling the reality that however skeletonic the financial crew may be in respect of numbers, it is in all other respects very much alive.

The details of performance need not be recounted here, since they are given on other pages, but a general conclusion can be drawn from them. It is that despite too small a number of technically-qualified people in charge; despite too heavy a load of work and responsibility; despite mechanical and logistical systems of accounting more appropriate to the 1920's than to the 1980's; and despite an ever-increasing burden of paperwork imposed by governments, in their bureaucratic interpretation of "accountability," the M.B.L. has made steady progress toward putting and keeping its fiscal house in order.

As the data show, we have escaped from the destructive cycle of operating deficits. Our intention is to avoid a return to that pattern, insofar as it is humanly possible, by means of strict financial self-discipline. Self-discipline has *not* brought with it the imposition of alien interests or rules and regulations foreign to the traditions of this Laboratory. It has interfered only slightly, if at all, with the style of all MBL administrations, past and current: to make possible the doing and teaching of highest-quality science with an absolute minimum of administratively generated distraction.

In a time when the most estimable universities and programs are suffering dislocations due to shortages of grant and contract support, the MBL has experienced, since 1977, a mean rise of about $275,000 per year in direct costs awarded. That is an average yearly increment in direct grant support of about 20%, which is not only opposite to national trend, but is also ahead of the rate of cost inflation. Improvements in the Laboratory's cost recovery position have made the results even better for *indirect* costs, and that is, in significant measure, the basis for the great improvement in the Laboratory's financial position.

Such support speaks to the quality of MBL *programs*, of course. Even in awards for training, so much in decline and so gravely threatened for the future everywhere, the MBL has done well: the Physiology course NIH grant was in it 21st continuous year, and continuing support came from other NIH institutes for Embryology and Neurobiology. The Rockefeller and Edna McConnell Clark Foundations made possible, with generous aid, the first offering of what has proved to be a brilliant new summer teaching program—our course in the Biology of Parasitism. New support has come from NASA, and generous help from the Grass Foundation and other private-sector donors has continued. The results speak also, however, to the quality of effort put forth by the skeleton crew referred to earlier. However superlative the substance of our programs is, they could not survive in the current financial and political climate without management that satisfies the federal authorities, the peer reviewers, and our own scholars and teachers.

The MBL system of financial control and operations is far from perfect: indeed, it is, in a structural and manpower sense, still quite inadequate. That causes us concerns (some of which the Treasurer refers to) for the future. But the concern is not ours alone. We should be concerned for the whole of our national enterprise in basic science. In the meantime, the MBL has done well—with the advice and very practical help of its cognizant Committees—in a domain of operations that is not so well done elsewhere. We have every right to be proud and to face the coming budget reductions squarely and with confidence.

Physical plant

All the major improvements of plant included within Phase One, Part I of the Second Century Fund Campaign are on, or ahead of schedule at the time of writing. These long awaited additions—a physical center for environmental sciences, rehabilitation of the Lillie Laboratory with expansion and improvement of the Library, restoration of the Candle House to serve as an administrative center, new and better storage facilities—are a part of the story of our capital campaign, which is better told elsewhere. They involve the work, on a large scale, of outside contractors, by which token they are not quite properly considered as "operations" of the MBL.

To the extent that such work needs oversight and careful management, however (and it needs those very much), it *is* an operational matter. Suffice it to say that by our traditional method—the assumption of extra work by staff already fully occupied with routine responsibility—these very sizeable building projects have been initiated and maintained on a reasonable schedule.

Meanwhile, the definitive "operations" have not suffered. The list of important special jobs completed during the past year is impressive. It covers three pages, closely typed, and represents direct expenditures of some $76,000. Included therein are such improvements as: completion of Whitman Building drainage upgrading; replacement of heaters and of furniture, as needed, in Memorial Circle cottages; major improvements to masonry work of the brick Apartment House, and the replacement there of 69 storm windows; rebuilding of the sea water pump house; repairs to the former Crane (now MBL) property on Millfield Street; complete remodelling of Lillie rooms 118–120 (A–F) as a teaching laboratory for the Parasitism course; replacement of underground services to the Marine Resources buildings and the Candle House; relocation of the Lillie lobby bulletin board, and construction of an attractive capital campaign display.

Important additions to this (partial) listing would be several critical *emergency* repairs and improvements of the kind that must, inevitably, be made in any physical plant as complex as ours. Notable among them this year were repairs to the electrical power system and to the sea water delivery system, which suffered from a prolonged interval of severe cold. It is to be stressed that *all* the above were undertakings separate from the routine and recurring plant operations of Buildings and Grounds and other Departments.

Research services

Quality and variety of services and facilities provided to investigators and to those participating in courses have continued to increase, as they have during the several years past. It is to the credit of the people who run those services that improvement, rather than decline, has accompanied budgetary stringency *and* an unprecedented level of occupancy (crowding might be a better term) during the summer of 1980.

There were, moreover, several small but not unappreciated immprovements in the quality of working life, introduced in 1980 at the initiative of this Department. They included the establishment of a typing service and a sharp decrease of turnaround time for work done in the MBL Photo Lab, without any loss of the customary quality. The Apparatus Department came through its most demanding summer without a single serious complaint from among the scores of people requiring its services and the use of its limited, but carefully maintained research equipment. Purchasing services and the general use facilities, including electron microscopy and hot labs, were at the usual level of high efficiency, one result of which is that they are held up, by many MBL investigators, to their home institutions as models.

Marine Resources

With a minimum of fuss, the 26 regularly used species (and, perhaps, 200 others) were collected and delivered to investigators, to courses, and to outside users. Some 100,000 animals, in all, were provided—nearly 62,000 of them to in-house summer experimenters. As a single-species example, more than 12,000 live adult squid were provided to several hundred investigators, usually within one day of the order or on standing order. Forty different laboratories used *Loligo pealei*, with total daily deliveries averaging 200 animals. Six teams of two men were charged with getting the animals from dockside to the place of work. The mean time interval for this task was 20 minutes, and losses of (the very fragile) squid in this stage of their delivery were negligible.

For such a record to be made and maintained by a quite small group of experts and dedicated helpers, housed in ancient buildings of inadequate design and capacity, is remarkable. Were it not that a new Marine Resources Center at the MBL is owed, first and foremost, to American Biology, we would be justified in arguing that it is owed to the members of the Marine Resources Department, and to those Corporation members who advise it.

Floating real estate of the Department, at least, will by the summer of 1981 have a very positive discontinuity: our new collecting vessel, R/V GEMMA, will be in full and daily operation. She and most of the other boats that do the Department's sea-borne work will be in a condition consistent with the importance and scope of the task. Getting the shoreside facilities into like condition is the urgent goal of Phase Three of our capital campaign.

Laboratory administration and the Department staff are not simply waiting, however. Active planning for buildings and for fleet improvements is in progress, and the needs for animal maintenance in *all* laboratory buildings are under careful study. There are, furthermore, active efforts underway (including proposals for planning and start-up support now in review) to systematize and consolidate the MBL's diverse programs in mariculture and marine animal medicine. These programs, quite aside from their inherent scientific quality and interest, are essential adjuncts to all Marine Resources efforts of the future. Many members of the MBL community concerned with them are involved in the planning.

Library

In August of 1980 the Library Committee completed its two-year study and issued an important report to the Corporation. Among the recommendations, which were accepted and approved along with the report entire, were the establishment of a joint management committee of the MBL and the WHOI, replacement of the existing committee with a small Users' Committee, and a number of quite specific recommendations on operations and improvements.

These recommendations were based in part upon the results of a questionnaire, mailed originally to 2500 users of the Library, to which an extraordinary 42% responded. That response will continue to be useful for some time, since sufficient information was elicited by the questionnaire not only to answer several urgent policy questions posed by the committee, but also to help set goals and priorities for future improvements of service and holdings.

All major recommendations of the report will have been implemented before the summer of 1981. It is fair to say that a higher level of cooperation in library management *and support* exists today, as between the MBL and the other Woods Hole institutions, most notably the Oceanographic, than ever before. Every indication is that cooperation will increase in the future, without any threat to the quality, nor to the historic styles and policies, of our much-admired Library.

The book collection was one of the few components of the Library singled out, by a significant number of users, for urgent improvement. Accordingly, a system of Subject Advisory Committees has been formed and is in operation, and a significant sum of money (most of it from a major foundation grant) has been budgeted for the current and the following year.

Architectural studies on physical changes to be made, in connection with the Lillie Laboratory rehabilitation, are in the meantime proceeding apace. These have been much advanced by the energetic advice of a distinguished outside librarian, Dr. Alan Erickson, who serves as consultant to the joint management committee, and has worked closely with the architects charged with making a survey-plan.

Public relations, fund raising

The public relations office, a relatively new MBL activity and under still newer management since October, 1980, is the source of a variety of communications with the public, as well as with Corporation Members and Associates. The magnitude *and* the quality of its effort have risen notably during the past year.

The task is a larger one than may be appreciated. Some 35 press releases, for example, were issued in 1980. New contacts with persons of responsibility in the media were initiated, and working relationships with the public relations people of other Woods Hole institutions were consolidated. Newspapers, magazines, and technical publications are monitored constantly for material relevant or potentially useful to the laboratory, especially in its news releases and fund raising efforts. Almost all requests for *general* information about the MBL pass through this office, and it is here that arrangements for tours, visits, and institutional participation of the Laboratory in meetings and other events, local and distant, are finalized.

The public relations office produces the biweekly calendar of lectures and other events as well as the two regular, non-technical house publications, *Nexus* and the *MBL Newsletter*. Special publications, *e.g.*, invitations, announcements, flyers, feature articles, and, of course, fund-raising materials such as the handsome new color brochure, are here assembled. Address lists are controlled and modified as needed, and with these and other tools of mass communications, the office assists the MBL Associates in a direct way. MBL Day, our now annual open house event, is a collaborative effort of the Associates and the Public Relations Office.

Similarly, the office serves as liaison between the Laboratory and the organizers of our various scholarship funds and programs. In 1980 the public relations officer and assistants played a significant role in the completion and in showings of the new MBL film, the making of which was supported by a generous grant from the Exxon Education Foundation.

The process of fund raising in the private sector is, however, one in which good public relations play only a part. The very heartening recent success we have enjoyed in this difficult field is in no small part the result of effective working relations between the Director's office and the Laboratory's outside fund raising consultant firm. In the absence of logistical support that would normally be provided by an in-house development group, the work of our consultants, the Bruce Porter Company, has been important not merely for prospect studies and strategic planning (as in other institutions), but for actual day-to-day support of the entire effort.

It is difficult for those not familiar with fund raising operations to imagine the magnitude of the task. A parallel can be drawn for Corporation members who are familiar with research grant applications to government agencies. Most of us have a good idea of the time and effort involved in the initial proposal-making and in recurrent reporting for a typical single-laboratory support grant of, say, fifty to eighty thousand dollars.

The time and effort involved in making a proposal for support at a like level, from private sources, is the same or greater (particularly if it requires, as is usually the case, a number of personal visits to foundations or corporate offices).

In that context, most Corporation members will readily understand what is implied by the Laboratory's having received more than $3.5 million in private-sector gifts and pledges since the start of the Second Century Fund campaign in 1979. Certainly, therefore, our consultants have been effective; indeed, indispensable. The time is near, nevertheless, when we must establish that in-house capability in Development for which they have been so timely a substitute. How and when that will be done has been the subject of much discussion between the administration and the Trustees, especially the Executive Committee. Positive steps are quite certain to be taken starting in the summer of 1981.

Housing and Educational Services

No survey of operations would be adequate without discussion of these two vital areas of staff activity, nor could *their* survey be contained within the compass of a report such as this without the use of many more words than have already been set down. Fortunately, however, both areas have received regular mention in earlier Director's Reports, and require no further statement here than the assurance to Corporation members that all is well.

The generally high quality and exceptional value (in terms of cost to users) of MBL housing and dining facilities have been maintained, and are more widely known, outside the MBL family, each year. Hence the Swope Center is indeed now used the year round, and is often quite full even out of the summer season. Most major meetings are booked several years ahead.

As to educational services, including such responsible tasks as operation of the admissions office, issuing schedules and course descriptions, handling applications and correspondence associated with them, organizing and coordinating new forms of educational activity such as the Short Courses, the January semester, the Aquavet program, and the like—these continue to be handled effectively and with despatch. All the more laudable, therefore, is the fact that the same staff are responsible for nearly all the physical provisions, *e.g.*, space assignment, instrumentation, and supplies, of the uniquely laboratory-based MBL summer courses.

Educational programs

The latter brings me to the end of this brief survey of operations and to the single additional subject of this year's Director's report. Here I *shall* mention names as appropriate, as is the intention for next year's report, in which the emphasis will be, instead, upon research programs.

In 1980 four of the seven summer courses were either new *in toto* or had new leadership and faculties. The new Biology of Parasitism course, housed in its handsome suite within the Lillie Laboratory, was a great success and has received favorable notice throughout the communities of Parasitology and Tropical Medicine, not to mention the more traditional communities of interest with the MBL, from which many listeners to the lectures came. John and Roberta David and their colleagues, with the imaginative aid of MBL staff, were able to accomplish in the first offering of this complex and highly advanced program what it would take several years, and very favorable physical and financial circumstances, to accomplish almost anyplace else.

Rudy Raff assumed the leadership of Embryology, and John Hildebrand and Tom Reese that of Neurobiology. The new versions of both courses were received with enthusiasm by students and by the community at large. Improved funding, especially from NASA and the Foundation for Microbiology, and new teaching space, allowed a doubling in size of the Microbial Ecology course, in a transition year of dual leadership of Holger Jannasch and Harlyn Halvorson. This program is now firmly established as a regular MBL summer course, an achievement for which Jannasch deserves much credit, and upon which it is quite clear that Halvorson, the next instructor-in-chief, will build.

In their expected excellent form were Physiology, under K. E. Van Holde, Neural Systems and Behavior, under Ronald Hoy, and Marine Ecology, led by Ivan Valiela and John Teal. Van Holde's tenure was completed in 1980, a fact that ought not to pass unnoticed and without the Corporation's sincerest thanks for his energetic and inspiring leadership. There is no doubt that his influence will continue to be felt in other ways, just as is the influence of Edward Kravitz, past director of the Neurobiology course, and in many ways the standard-setter of that extraordinary offering. Van Holde is to be succeeded in 1981 by Joel Rosenbaum, and Hoy will be joined in 1981 by a co-director, Eduardo Macagno.

Lawrence Bogorad assembled, at the request of the Director, a distinguished *ad hoc* committee charged with a study of education in botany at the MBL. In due course the committee issued a report recommending the establishment of a new summer course, replacing the former Experimental Marine Botany program. In the new version, nitrogen metabolism and phytoplankton ecosystems will serve as organizing topics around which the high-level instruction is to be built. Several accomplished plant scientists who are potential faculty (including instructor-in-chief) of that course will be in residence during the summer of 1981, and the intention is to mount the full course in 1982.

The 1980 January Neurobiology course was reorganized under the imaginative leadership of Dan Alkon, while Walter Vincent (Developmental Biology) and George Woodwell (Ecology) ended successful terms as course directors that year, to be succeeded in 1981 by Kenneth Edds and an Ecosystems Center staff team, respectively. Jelle Atema (Behavior) and Fred Bang (Comparative Pathology) led their excellent courses as in prior years.

During the summer of 1981 there is to be a thorough review of the January offerings as a group, with a view to possible alternative arrangements of scheduling and level. Such a study, when as large a group of Instruction Committee members as possible can be assembled, is made urgent by a nationwide pattern of altered college and university calendars, one result of which is a declining enrollment in the MBL January courses (although not a declining *quality* of students).

There were 13 weeks of short course offerings in 1980, ably organized and facilitated by Morton Maser and his staff. These advanced, high-intensity technical offerings continue to be excellently subscribed, and to receive strong material support from the manufacturers of instrumentation.

Sources of support for students, very much threatened for the future by the likely decline of government training grants, were nevertheless adequate in 1980, particularly because of supplementation via the Founders' Scholarships (John and Elizabeth Buck and Isabel Mountain, coordinators) and other named scholarships. The Aline D. Gross scholarship fund grew sufficiently to insure that two outstanding young women will receive its support in the summer of 1981. The Macy Scholars program and the Steps Toward Independence program continued to provide support for a large group of students and young investigators who would otherwise not have the benefit of course attendance or research at the MBL.

Conclusion

The MBL is in very good health.

There is a miasma spreading over the landscape of education and research in biology, composed in equal parts of inflation and reduced support from agencies of government. In the noxious atmosphere, with its synergistic components, many excellent programs and even entire institutions have already sickened. It is an irony of fate for this Laboratory that is so important to us, and, indeed, to the scientific endeavor of this nation, to have been brought to a state of renewed vigor and confidence only to find itself exposed to a newly hostile atmosphere.

There is, on the other hand, a different and perhaps a more appropriate way to see it: Our Laboratory has had its operations, it educational program, and its research programs, year round and seasonal, strengthened *just in time* to face the threat, and to survive it without major blemish or loss.

There is every reason to look forward to the MBL's hundredth birthday year as one in which this, our national laboratory of biology in all but name, will be functioning at least as well as it did in 1980, and in physical surroundings vastly sounder and more beautiful than they were in that year.

VII. REPORT OF THE TREASURER

Beating against a continuing gale of inflation, the Laboratory nevertheless made excellent financial headway during 1980. Unrestricted revenue exceeded expenses by $323,000, compared to $31,000 in 1979. Even after recognition of the non-cash depreciation of the physical plant, $284,227, revenues more than covered expenses. Your Treasurer is most happy.

To put the financial year in perspective, I must point out that some of the increase in revenues in 1980 was not of an operating nature. Unrestricted gifts were up $190,000 over 1979. Investment income was 71 percent higher. Two factors entered into the latter result. The Laboratory had substantial sums earmarked for construction and renovation projects during the year, including a $900,000 gift from the Max C. Fleischman Foundation, and these funds were very profitably invested while awaiting their intended use. In addition, yields on other investments also were very strong during the high interest rate conditions prevailing throughout the year.

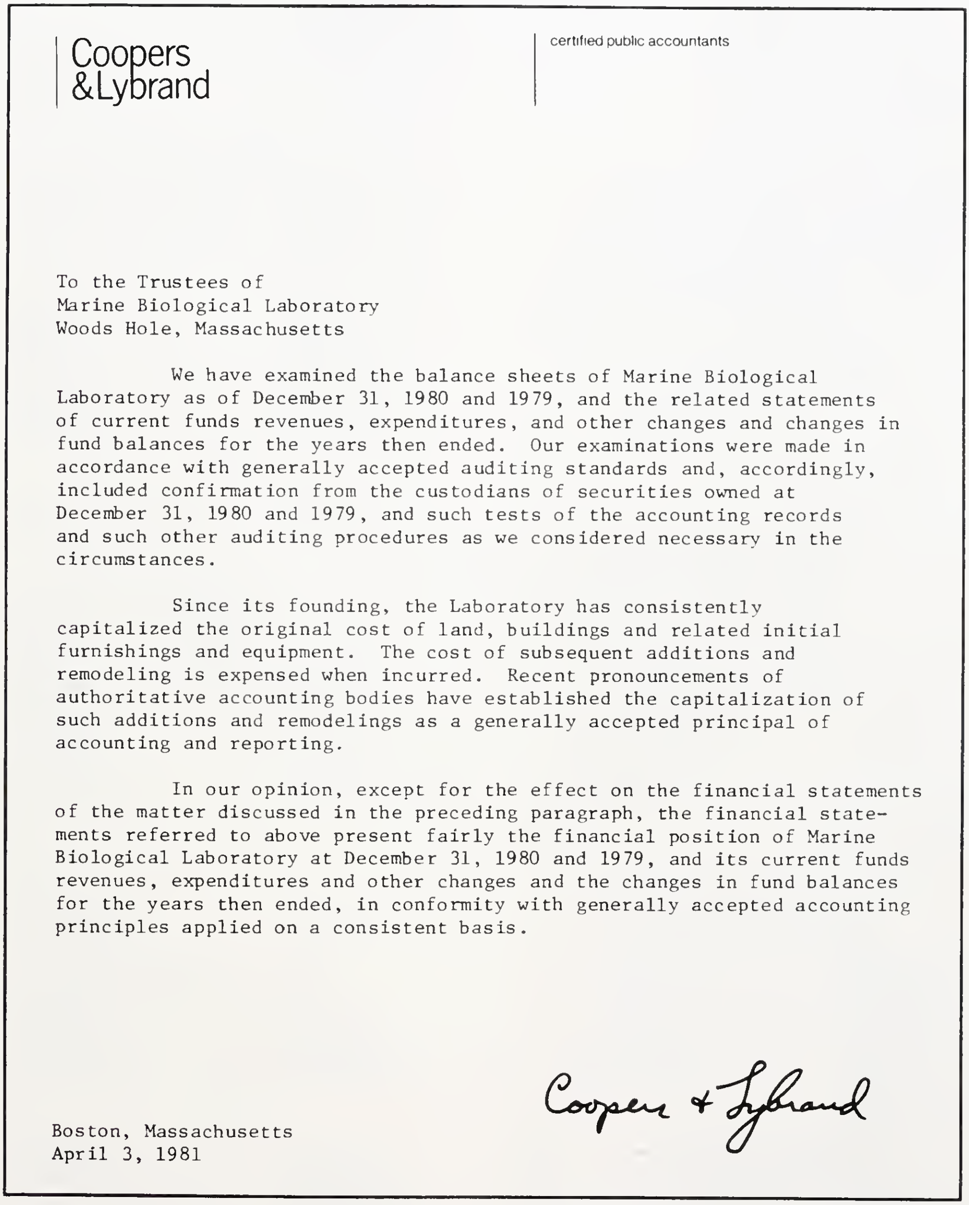
Notwithstanding my occasional carping about the need for greater budgetary discipline on the part of course directors, I was generally pleased with the financial aspects of the Laboratory's 1980 operations. A more equitable, government approved, overhead rate and more realistic charges for research space generated revenues of $1,226,000 in 1980, versus $929,000 in the prior year. Increases in various fees also helped offset rising costs.

Operating expenses increased $459,000, or 18 percent, over 1979's level. If one takes into consideration increased utilities expenses and the expenses associated with the performance of previously deferred maintenance (the two items summing to $97,000) and the increase in administrative expenses, which is largely attributable to the Second Century Campaign ($130,000), one sees that all other expense categories increased by $232,000. This increase of 13 percent over 1979 is very much in line with 1980's rate of inflation.

As a volunteer officer of the MBL, and one who is not also an investigator conducting research at the Laboratory, I have acquired a special appreciation for the integrity of those members of the Executive Committee who are part of MBL's scientific community. They have commendably balanced a concern for the financial health of the institution against the realization that their associates and their students bear the impact of decisions to increase fees, space charges, or cottage rentals. Clearly, the MBL's sociology ordinarily would give rise to a tendency to ignore the reality of inflation. Nevertheless, I perceive that the Executive Committee recognizes that virtually every vendor to the MBL has indexed prices to inflation, and that the Laboratory soon would be strangled by rising costs if such increases were not passed on promptly to the users of its facilities.

At this writing, we are four months into 1981. I believe we can be optimistic about the Laboratory's ability to meet its budgetary goals for this year. The longer view into 1982 is a bit more worrisome. Expected cutbacks in federal programs undoubtedly will have a direct effect on certain of our revenue categories. Indirectly, reductions in federal spending also may affect gifts and grants from foundations and other private sources, which will receive many more requests from organizations seeking to replace federal funding. Our greatest concern must be that revenues may dwindle more rapidly than the rate at which inflation declines (if, indeed, it does abate), producing a vicious potential for large and continuing deficits. We are alert to that possibility.

I am happy to present the auditor's report, which follows.



Coopers
&Lybrand

certified public accountants

To the Trustees of
Marine Biological Laboratory
Woods Hole, Massachusetts

We have examined the balance sheets of Marine Biological Laboratory as of December 31, 1980 and 1979, and the related statements of current funds revenues, expenditures, and other changes and changes in fund balances for the years then ended. Our examinations were made in accordance with generally accepted auditing standards and, accordingly, included confirmation from the custodians of securities owned at December 31, 1980 and 1979, and such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

Since its founding, the Laboratory has consistently capitalized the original cost of land, buildings and related initial furnishings and equipment. The cost of subsequent additions and remodeling is expensed when incurred. Recent pronouncements of authoritative accounting bodies have established the capitalization of such additions and remodelings as a generally accepted principal of accounting and reporting.

In our opinion, except for the effect on the financial statements of the matter discussed in the preceding paragraph, the financial statements referred to above present fairly the financial position of Marine Biological Laboratory at December 31, 1980 and 1979, and its current funds revenues, expenditures and other changes and the changes in fund balances for the years then ended, in conformity with generally accepted accounting principles applied on a consistent basis.

Coopers & Lybrand

Boston, Massachusetts
April 3, 1981

MARINE BIOLOGICAL LABORATORY

BALANCE SHEETS

December 31, 1980 and 1979

Assets	*1980*	*1979*
Current Funds:		
Unrestricted:		
Cash, including deposits at interest	$ 337,867	$ 424,763
Money market securities	1,250,000	500,000
Accounts receivable, net of allowance for uncollectible accounts of $8,000 in 1980 and $1,716 in 1979	1,462,042	858,466
Other assets	5,004	11,186
Due to restricted current funds	(1,656,041)	(729,275)
Due to invested funds	(25,139)	(8,151)
Total unrestricted	1,373,733	1,056,989
Restricted		
Cash	24,510	12,285
Investments, at cost: market value: 1980—$2,123,541; 1979—$1,881,590 (Notes B and F)	2,085,227	1,900,292
Due from unrestricted current fund	1,656,041	729,275
Due from invested funds	350,967	350,967
Total restricted	4,116,745	2,992,819
Total current funds	$5,490,478	$4,049,808
Invested Funds:		
Cash	1,530	5,589
Investments, at cost; market value: 1980—$4,712,945; 1979—$4,250,838 (Notes B and F)	4,219,999	3,936,137
Due from unrestricted current fund	25,139	8,151
Due to restricted current funds	(350,967)	(350,967)
Total invested funds	$3,895,701	$3,598,910
Plant Fund:		
Land, buildings and equipment at cost (Note C)	12,940,384	12,258,651
Less accumulated depreciation	4,535,825	4,251,598
Total plant fund	$ 8,404,559	$ 8,007,053

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

BALANCE SHEETS

December 31, 1980 and 1979

Liabilities and Fund Balances	*1980*	*1979*
Current Funds:		
Unrestricted:		
Accounts payable and accrued expenses	$ 490,305	$ 268,369
Deferred income	75,489	68,006
Fund balance	807,939	720,614
Total unrestricted	1,373,733	1,056,989
Restricted:		
Fund balances:		
Unexpended gifts and grants	4,027,352	2,897,679
Unexpended income of endowment funds	89,393	95,140
Total restricted	4,116,745	2,992,819
Total current funds	$5,490,478	$4,049,808
Invested Funds:		
Endowment funds	2,077,500	1,918,170
Quasi-endowment funds	934,143	934,143
Retirement fund (Note D)	884,058	746,597
Total invested funds	$3,895,701	$3,598,910
Plant Fund:		
Invested in plant	8,404,559	8,007,053
Total plant fund	$8,404,559	$8,007,053

The accompanying notes are an integral part of the financial statements

MARINE BIOLOGICAL LABORATORY

STATEMENTS OF CURRENT FUNDS REVENUES, EXPENDITURES, AND OTHER CHANGES

for the years ended December 31, 1980 and 1979

	Unrestricted		*Restricted*		*Total*	
	1980	*1979*	*1980*	*1979*	*1980*	*1979*
Revenues:						
Instruction:						
Tuition	$ 260,949	$ 238,378	$ 31,500	$ 15,500	$ 292,449	$ 253,878
Grants and contracts:						
Government	15,465	19,788	306,465	215,719	321,930	235,507
Private	22,000		192,224	53,887	214,224	53,887
Research:						
Laboratory rentals	723,986	565,631			723,986	565,631
Grants and contracts:						
Government	397,152	283,770	1,589,736	1,304,680	1,986,888	1,588,450
Private	104,530	79,468	134,709	225,887	239,239	305,355
Dormitory	386,600	319,010			368,600	319,010
Dining Hall	188,232	179,507			188,232	179,507
Library	133,728	130,609			133,728	130,609
Biological Bulletin	98,877	87,416			98,877	87,416
Support departments:						
Research services	259,834	220,914			259,834	220,914
Marine resources	80,332	92,277			80,332	92,277
Investment income	292,722	171,215	289,672	188,641	582,394	359,856
Gifts	304,045	114,520	89,496	120,178	393,541	234,698
Other	142,828	139,491			142,828	139,491
Total revenues	3,393,280	2,641,994	2,633,802	2,124,492	6,027,082	4,766,486

Operating expenditures:						
Instruction	264,238	214,687	326,216	132,387	590,454	347,074
Research	1,726	(7,019)	1,945,869	1,675,172	1,947,595	1,668,153
Scholarships and stipends			295,937	236,958	295,937	236,958
Dormitory	165,688	143,794			165,688	143,794
Dining Hall	178,810	169,604			178,810	169,604
Library	272,261	252,713	49,784	36,089	322,045	288,802
Biological Bulletin	114,628	84,082			114,628	84,082
Support departments:						
Research services	392,422	355,719		5,000	392,422	360,719
Marine resources	221,250	226,690			221,250	226,690
Administration	697,270	567,396	13,296	36,314	710,566	603,710
Plant operation	761,515	602,969	2,700	2,572	764,215	605,541
Total expenditures	3,069,808	2,610,635	2,633,802	2,124,492	5,703,610	4,735,127
Excess of revenues	323,472	31,359	—	—	323,472	31,359
Transfers and additions:						
Excess of restricted gifts, grants and investment income received over amounts expended			1,123,926	1,137,274	1,123,926	1,137,274
From plant fund—proceeds of sale of plant assets		144,700				144,700
Additions to buildings and equipment	(236,147)				(236,147)	
Net transfers and additions	(236,147)	144,700	1,123,926	1,137,274	887,779	1,281,974
Net increase in fund balances	$ 87,325	$ 176,059	$1,123,926	$1,137,274	$1,211,251	$1,313,333

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

STATEMENTS OF CHANGES IN FUND BALANCES

for the years ended December 31, 1980 and 1979

	Current Funds		Invested Funds			
1979	*Unrestricted*	*Restricted*	*Endowment*	*Quasi-Endowment*	*Retirement*	*Plant Fund*
Balances at December 31, 1978	$ 544,555	$1,855,545	$2,174,027	$934,143	$618,600	$8,361,522
Increases:						
Unrestricted current fund revenues	2,641,994					
Grants and gifts		3,422,789				
Investment income		206,503			38,148	
Proceeds of sale of equipment						144,700
Addition to pension fund					113,062	
Tuition		15,500				
Decreases:						
Instruction, research and general expenditures	(2,610,635)	(2,124,492)				
Indirect costs		(383,026)				
Realized net losses on sale of investments			(255,857)			
Payments to pensioners					(23,213)	
Net book value of equipment sold						(76,455)
Depreciation						(278,014)
Transfers—additions (deductions):						
Proceeds of sale of equipment	144,700					(144,700)
Balances at December 31, 1979	720,614	2,992,819	1,918,170	934,143	746,597	8,007,053

	Current Funds		Invested Funds			
1980	*Unrestricted*	*Restricted*	*Endowment*	*Quasi-Endowment*	*Retirement*	*Plant Fund*
Increases:						
Unrestricted current fund revenues	3,393,280					
Grants and gifts		3,955,840				
Investment income		309,535			44,904	
Realized net gains on sale of investments			159,330			
Additions to buildings, from restricted gifts						445,586
Addition to pension fund					117,557	
Tuition		31,500				
Decreases:						
Instruction, research and general expenditures	(3,069,808)	(2,633,802)				
Indirect costs		(539,147)				
Realized net losses on sale of investments					(671)	
Payments to pensioners					(24,329)	
Depreciation						(284,227)
Transfers—additions (deductions):						
Additions to buildings and equipment	(236,147)					236,147
Balances at December 31, 1980	$ 807,939	$4,116,745	$2,077,500	$934,143	$884,058	$8,404,559

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

NOTES TO FINANCIAL STATEMENTS

A. *Purpose of the Laboratory:*

The purpose of Marine Biological Laboratory (the "Laboratory") is to establish and maintain a laboratory or station for scientific study and investigations, and a school for instruction in biology and natural history.

B. *Significant Accounting Policies:*

Basis of Presentation—Fund Accounting

In order to ensure observance of limitations and restrictions placed on the use of resources available to the Laboratory, the accounts of the Laboratory are maintained in accordance with the principles of "fund accounting." This is the procedure by which resources are classified into separate funds in accordance with activities or objectives specified. In the accompanying financial statments, funds that have similar characteristics have been combined.

Externally restricted funds may only be utilized in accordance with the purposes established by the source of such funds. However, the Laboratory retains full control over the utilization of unrestricted funds. Restricted gifts, grants, and other restricted resources are accounted for in the appropriate restricted funds. Restricted current funds are reported as revenue when expended for current operating purposes. Unrestricted revenue is reported as revenue in the unrestricted current fund when received.

Endowment funds are subject to restrictions requiring that the principal be invested and only the income utilized. Quasi-endowment funds have been established by the Laboratory for the same purposes as endowment funds; however, any portion of these funds may be expended.

Investments

Investments purchased by the Laboratory are carried at cost. Investments donated to the Laboratory are carried at fair market value at date received. For determination of gain or loss upon disposal, cost is determined based on the specific identification method.

Investment Income and Distribution

The Laboratory follows the accrual basis of accounting except that investment income is recorded on a cash basis. The difference between such basis and the accrual basis does not have a material effect on the determination of investment income earned on a year-to-year basis.

Investment income includes income from the investments of specific funds and from the pooled investment account. Income from the pooled investment account is distributed to the participating funds on the basis of the market value at the beginning of the quarter, adjusted for the cost of any additions or disposals during the quarter.

C. *Land, Buildings and Equipment:*

Following is a summary of the plant fund assets:

Classification	*1980*	*1979*
Land	$ 639,693	$ 639,693
Buildings	10,694,543	10,190,430
Equipment	1,606,148	1,428,528
	12,940,384	12,258,651
Less accumulated depreciation	4,535,825	4,251,598
	$ 8,404,559	$ 8,007,053

The original cost of land, buildings and related initial furnishings and equipment is capitalized when the assets are acquired. The cost of subsequent additions and purchases, repairs and remodeling is expensed when incurred. Equipment and remodeling expenditures amounted to approximately $135,000 and $76,000 in 1980 and 1979, respectively.

Depreciation is computed using the straight-line method over estimated useful lives of 40 years for buildings and 20 years for equipment.

D. *Retirement Fund:*

The Laboratory has a non-contributory pension plan for substantially all full-time employees which complies with the requirements of the Employee Retirement Income Security Act of 1974. The actuarially-determined pension expenses charged to operations in 1980 and 1979 were $117,557 and $113,062 respectively. The Laboratory's policy is to fund pension costs accrued, as determined under the aggregate level cost method. As of the latest valuation date, based on benefit information obtained January 1, 1981, the actuarial present values of vested and non-vested benefits, assuming an investment rate of return of 6%, were approximately $816,030 and $33,768 respectively. At January 1, 1981 net assets of the Plan available for benefits, were approximately $918,855.

E. *Pledges and Grants:*

As of December 31, 1980 and 1979, the following amounts remain to be received from previous gifts and grants for specific research and instruction programs, and are expected to be received as follows:

	December 31, 1980		*December 31, 1979*	
	Unrestricted	*Restricted*	*Unrestricted*	*Restricted*
1980			$ 50,500	$3,689,270
1981	$104,000	$1,061,356	49,000	215,000
1982	50,000	66,333	15,000	
1983	5,000			
	$159,000	$1,127,689	$114,500	$3,904,270

F. Investments:

The following is a summary of the cost and market value of investment assets at December 31, 1980 and 1979, and the related investment income and disposition of investment income for the years ended December 31, 1980 and 1979.

	Cost		*Market*		*Investment Income*	
	1980	*1979*	*1980*	*1979*	*1980*	*1979*
Invested Funds:						
U. S. Government securities	$ 910,077	$ 986,397	$ 814,012	$ 930,834	$ 88,349	$ 88,030
Corporate fixed income obligations	878,062	1,162,164	744,161	1,038,929	96,609	64,683
Common stocks	2,414,311	1,705,817	3,137,223	2,213,951	133,246	142,530
Preferred stocks		64,210		49,575	2,033	2,976
Units in combined pension fund						(1,719)
Real estate (market at cost)	17,549	17,549	17,549	17,549		
Total	$4,219,999	$3,936,137	$4,712,945	$4,250,838	320,237	296,500
Less custodian fees					25,105	23,794
					295,132	272,706

	Cost		*Market*		*Investment Income*	
	1980	*1979*	*1980*	*1979*	*1980*	*1979*
Current Restricted Funds:						
U. S. Government securities	$1,113,603	$1,537,142	$1,044,597	$1,523,690	134,378	112,245
Corporated Fixed Income Obligation	337,550	363,150	325,663	357,900	47,249	9,272
Common Stocks	634,074	—	753,281	—	17,806	—
	$2,085,227	$1,900,292	$2,123,541	$1,881,590	199,433	121,517
Less custodian fees					5,992	2,810
					193,441	118,707
Current Unrestricted Funds:						
Earned on corporate savings accounts and money market funds					158,588	24,453
Investment income					$647,161	$415,866

Disposition of Investment Income:

	1980	1979
Restricted for current use:		
Utilized in current operations	$289,672	$188,641
Available for future operations	19,863	17,862
Total restricted current funds	309,535	206,503
Retirement fund	44,904	38,148
Unrestricted—utilized in current operations	292,722	171,215
	$647,161	$415,866

VIII. REPORT OF THE LIBRARIAN

During August of 1980 the Library Committee completed its 2-year study with the following proposal to the Director and the Corporation:

1. A Joint Management Committee should be established, consisting of the Directors of MBL and WHOI together with a small number of additional administrative and scientific personnel. This Committee would set operating policies for the library, establish its budget, and direct an active fund-raising campaign.
2. The joint library should continue to be operated by MBL.
3. A Users Committee should be established to advise and guide the Joint Management Committee. This Users Committee should be the MBL Standing Committee on the Library.

Following a request by the Director to "assay the level of user satisfaction," the Library Committee prepared and mailed a comprehensive questionnaire to 2400 past and present users. One thousand responses were received within 6 weeks. A summary of the results follows:

1. The library was considered equal to or better than a "home" library by 70 to 90% of the users with respect to periodical collection, services, organization, and physical facilities. In contrast, only 48% found the book collection better than other libraries. Highest priority for expenditure of future funding was assigned to (1) periodical collection—63%, (2) book collection—50%, (3) new services—12%, and (4) physical facilities—9%.
2. The majority of users favored establishing cooperative management and fund-raising machinery with the WHOI (78%) while keeping the library an MBL operation, as opposed to forming a separate jointly operated library corporation (21%) or two separate libraries (1%).

A complete analysis of the results of the questionnaire is available on request. The book collection received much needed attention and money this year. Subject committees were formed with scientists from all four institutions in Woods Hole, and recommendations and purchases will be made during 1981.

Ann White, our Loan Librarian and dear friend, died in October. The *Woods Hole Passage* carried the following comments by Suzanne Volkmann: "Ann was one of those very special people we are sometimes privileged to know—a truly good person who was faithful to her convictions . . . she kept things in perspective, enjoying a challenge by not expecting too much, seeking the positives but not brooding over the negatives. Her knowledge was in balance with her humility, her ready wit with her compassion." We miss her.

IX. EDUCATIONAL PROGRAMS

SUMMER

BIOLOGY OF PARASITISM

Instructor-in-chief

DAVID, JOHN R., Harvard Medical School

Other faculty, staff, and lecturers

ASKENASE, PHILIP, Yale University School of Medicine
BANG, FREDERIK, Johns Hopkins University
BROOKS, DANIEL, National Zoological Park, Smithsonian Institute
CAULFIELD, JOHN, Harvard Medical School
CANTOR, HARVEY, Harvard Medical School
CARTER, RICHARD, National Institutes of Health
CERAMI, ANTHONY, Rockefeller University
CHERNIN, ELI, Harvard School of Public Health
CROSS, GEORGE, Wellcome Research Laboratories, England, U. K.

David, Roberta, Harvard Medical School
Fearon, Douglas, Harvard Medical School
Gitler, Carlos, The Weizmann Institute, Israel
Goodenough, Daniel, Harvard Medical School
Guerrant, Richard, University of Virginia School of Medicine
Hommel, Marcel, Harvard Medical School
Karnovsky, Manfred, Harvard Medical School
Mahmoud, Adel, Case Western Reserve University
McMahon Pratt, Diane, Harvard Medical School
Michelson, Edward, Harvard School of Public Health
Miller, Louis, National Institutes of Health
Nussenzweig, Ruth, New York University School of Medicine
Pfefferkorn, Elmer, Dartmouth Medical School
Piessens, Willy, Harvard Medical School
Rifkin, Mary, Rockefeller University
Roberts, Bryan, Harvard Medical School
Sher, Alan, Harvard Medical School
Sherman, Irwin, University of California, Riverside
Spielman, Andrew, Harvard School of Public Health
Trager, William, Rockefeller University
Unanue, Emil, Harvard Medical School
Waksman, Bryon, National Multiple Sclerosis Society
Warren, Kenneth, Rockefeller Foundation
Weller, Thomas, Harvard School of Public Health

Students[1]

*Anderson, Sally, University of Texas Health Science Center
*Arias-Negrete, Sergio, Centro de Investigación y De Estudios Avanzados, Mexico
*Boutte, Kenneth, University of California, Berkeley
*Canlas, Manuel, University of the Philippines, Philippines
*Hafez, Khalil, Biomedical Research Center for Infectious Diseases, Egypt
*Johnson, Barbara, Colorado State University
*Johnston, Michael, Massachusetts Institute of Technology
*Jones, Rena, Spelman College
*Klass, Perri, University of California, Berkeley
*Klotz, Francis, University of Alabama
*Koech, Davy, University of Nairobi Medical School, Kenya
*Lynch, Eileen, Albert Einstein College of Medicine
*Mellen, Paul, Boston University
*Rosenberg, Ian, Weizman Institute, Israel
*Tumboh-Oeri, Aloys, Rockefeller University
*Wirth, Dyann, Harvard University

Embryology

Instructor-in-chief

Raff, Rudolf A., Indiana University

Other faculty, staff, and lecturers

Arnold, John, University of Hawaii
Begg, David, Harvard Medical School
Brandhorst, Bruce, McGill University, Canada
Brun, Rudolf, Texas Christian University
Dan, Katsuma, Tokyo, Japan

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[1] All summer students listed completed the formal course programs. Asterisk indicates those completing post-course research sessions.

Davidson, Eric, California Institute of Technology
Epel, David, Stanford University
Ernst, Susan, Tufts University
Firtel, Richard, University of California, San Diego
Gould, Stephen Jay, Harvard University
Gross, Paul R., Marine Biological Laboratory
Harkey, Michael, University of Washington
Horwitz, Robert, Massachusetts Institute of Technology
Humphreys, Tom, University of Hawaii
Hunt, Tim, Cambridge University, England, U. K.
Ilan, Joseph, Case Western Reserve University
Infante, Anthony, Wesleyan University
Inoué, Shinya, Marine Biological Laboratory/University of Pennsylvania
Jaffee, Lionel, Purdue University
Jeffery, William, University of Texas
Katz, Michael, Case Western Reserve University
Kauffman, Stuart, University of Pennsylvania
Kaufman, Thomas, Indiana University
Keller, Raymond, University of California, Berkeley
Kirschner, Marc, University of California, San Francisco
Klein, William, Indiana University
Newrock, Kenneth, McGill University, Canada
Okazaki, Kayo, Tokyo Metropolitan University, Japan
Rosbach, Michael, Brandeis University
Ruderman, Joan, Harvard Medical School
Rustad, Ronald, Case Western Reserve University
Sargent, Thomas, California Institute of Technology
Spiegel, Evelyn, Dartmouth College
Spradling, Allan, Carnegie Institution of Washington
Tamm, Sidney, Boston University Marine Program
Trinkaus, J. P., Yale University
Vacquier, Victor, Scripps Institution of Oceanography
White, Shirley, Woods Hole, Massachusetts
Whittaker, J. R., Wistar Institute

Students[1]

*Bédard, André, McGill University, Canada
*Bruskin, Arthur, Indiana University
*Compton, Reid, University of Virginia
*Crews, David, Harvard University
*De Santis, Rosaria, Stazione Zoologica di Napoli, Italy
*Gimlich, Robert, University of California, Santa Barbara
*Glick, Adam, Oberlin College
*Goff, Stephen, Harvard University
*Gordon, Katherine, Wesleyan University
*Huck, Kathleen, Johns Hopkins University
*Hursh, Deborah, Indiana University
*Kirby, Brooke, University of California, Irvine
*Laufer, John, University of Colorado
*Leaf, David, Indiana University
*Madore, Mae, University of Vermont
*Mendel, Eileen, Wesleyan University
*Monk, Peter, Rutgers University
*Mortin, Mark, Indiana University
*Olsen, Cherie, Ohio State University
*Roth, Mark, University of Colorado

*SEITZ, ANNA, University of Pennsylvania
*SERVETNICK, MARC, University of California, Berkeley
*STOPAK, DAVID, University of North Carolina
*WADHWANI, KISHENA, University of Maryland

MARINE ECOLOGY

Instructors-in-chief

TEAL, JOHN, Woods Hole Oceanographic Institution
VALIELA, IVAN, Boston University/Marine Biological Laboratory

Other faculty, staff, and lecturers

CAMPBELL, MIKE, National Marine Fisheries Service
CAPUZZO, JUDITH, Woods Hole Oceanographic Institution
CARACO, NINA, Marine Biological Laboratory and Boston University
CARLTON, JIM, Woods Hole Oceanographic Institution
COWLES, TIM, Woods Hole Oceanographic Institution
DACY, JOHN, Woods Hole Oceanographic Institution
FORD, TIM, Sussex University, England, U. K.
GOLDMAN, JOEL, Woods Hole Oceanographic Institution
GRASSLE, FRED, Woods Hole Oceanographic Institution
GRICE, GEORGE, Woods Hole Oceanographic Institution
HAIBACH, MARK, University of Michigan
HOBBIE, JOHN, Marine Biological Laboratory and Boston University
HUMES, ARTHUR, Marine Biological Laboratory and Boston University
JACKSON, JEREMY, Johns Hopkins University
JANNASCH, HOLGER, Woods Hole Oceanographic Institution
JEFFERIES, BOB, University of Toronto, Ontario, Canada
KAUFMANN, CARL, Johns Hopkins University
KOEHL, MIMI, University of California, Berkeley
LADERMAN, AIMLEE, State University of New York, Binghampton
LEIGHTY, BRUCE, Dartmouth College
MADIN, L., Woods Hole Oceanographic Institution
MANN, ROGER, Woods Hole Oceanographic Institution
NIXON, SCOTT, University of Rhode Island
ODUM, BILL, University of Virginia
PETERSON, BRUCE, Marine Biological Laboratory

Students[1]

*ALLENDE, GLORIA, Universidad Nacional de Cordoba, Argentina
ASHENFELTER, MARK, Harvard University
*BENNETT, VIRGINIA, University of California, Santa Cruz
BOYCE, THOMAS, Vassar College
*CHAMBERS, RANDOLPH, University of Massachusetts
CORR, MICHELLE, University of Oklahoma
DISE, NANCY, University of Notre Dame
*FUJITA, RODNEY, Boston University
GROESBECK, ROBERT, Oberlin College
*HORGAN, ERICH, Hartwick College
HUGHES, DAVID, University College of North Wales, Wales, U. K.
*JACKSON, JAMES, Oberlin College
*LEIGHTY, BRUCE, Dartmouth College
LIGHT, DAVID, Chappaqua, New York
*MANSOUR, RANDA, University of North Carolina
MOLESWORTH, JENNIFER, Plattsburgh State University College

Pires, Anthony, Harvard University
Scheuer, Debbie, University of Hawaii
*Sherrill, Dorothy, Auburn University
*Spies, Annette, University of Washington
*Thomson, James, University of Illinois, Champaign-Urbana
Usem, Michael, Stanford University
*Uyeki, Timothy, Oberlin College
Wilcox, Thomas, Hampden-Sydney College

Microbial Ecology

Instructors-in-chief

Jannasch, Holger W., Woods Hole Oceanographic Institution
Halvorson, Harlyn O., Brandeis University (on sabbatical)

Other faculty, staff, and lecturers

Canale-Parola, Ercole, University of Massachusetts, Amherst
Carpenter, Edward J., State University of New York, Stony Brook
Castenholz, Richard W., University of Oregon, Eugene
Cohen, Yehuda, Hebrew University, Steinitz Marine Laboratory, Elat, Israel
Cuhel, Russell L., Woods Hole Oceanographic Institution
Gibson, Jane A., Cornell University, Ithaca
Goldman, Joel C., Woods Hole Oceanographic Institution
Gottschal, Jan C., University of Groningen, Holland
Greenberg, Everett P., Cornell University
Hanson, Richard S., University of Wisconsin, Madison
Howarth, Robert W., Marine Biological Laboratory
Jørgensen, Bo Barker, University of Aarhus, Denmark
Kuenen, Gijs J., University of Groningen, Holland
Leadbetter, Edward R., University of Connecticut
Lee, John J., City University of New York, City College
Matin, Abdul, Stanford University
Molongoski, John J., Woods Hole Oceanographic Institution
Peck, Harry D., University of Georgia, Athens
Poindexter, Jeanne S., Public Health Research Institute, New York
Ruby, Edward G., Woods Hole Oceanographic Institution
Scranton, Mary, State University of New York, Stony Brook
Taylor, Craig D., Woods Hole Oceanographic Institution
Vincent, Walter S., University of Delaware, Newark
Waterburg, John B., Woods Hole Oceanographic Institution
Wolfe, Ralph S., University of Illinois, Urbana

Students[1]

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*Brandl, Helmut, Universitat Zurich, Switzerland
*Campbell, Lisa, State University of New York, Stony Brook
*Chin, Edward, Jr., University of Georgia
*Clancy, Timothy P., University of Vermont
*Coleman, James P., North Carolina State University
*Crill, Patrick M., University of North Carolina
*Dill, Therese, University of Maryland
*Fitzgerald, Kathleen A., University of Connecticut
Kropinski, Andrew M., Queen's University, Canada
Lavoie, Kathleen H., University of Illinois at Chicago Circle
Lehman, Nancy K., Fort Hays State University

*Lerud, Rebecca F., University of Bridgeport
*Lessard, Evelyn, University of Rhode Island, Kingston
Mehrotra, Bharati, Tougaloo College
*Mollura, Francesca, LeMoyne College
Odum, J. Martin, University of Georgia
Paterek, James R., University of Florida, Gainesville
Seftor, Richard E. B., University of California, Los Angeles
*Terracciano, Joseph, University of Massachusetts, Amherst
*Weber, Frederick H., Northeastern University

Neural Systems and Behavior

Instructor-in-chief

Hoy, Ronald R., Cornell University

Other faculty, staff, and lecturers

Bentley, David, University of California, Berkeley
Calabrese, Ronald, Harvard University
Derby, Charles, Boston University
Eisner, Thomas, Cornell University
Erber, Joachim, Free University of Berlin, W. Germany
Gelperin, Alan, Princeton University
Hall, Jeffrey, Brandeis University
Hall, Linda, Albert Einstein College of Medicine
Horvitz, Howard R., Massachusetts Institute of Technology
Kalmijn, Adrianus, Woods Hole Oceanographic Institution
Kalmijn, Vera, Woods Hole Oceanographic Institution
Kankel, Douglas, Yale University
Keeton, William, Cornell University
Kelley, Darcey, Princeton University
Kristan, William, University of California, San Diego
Levinthal, Cyrus, Columbia University
Levinthal, Francois, Columbia University
Macagno, Eduardo, Columbia University
Milde, Juergen, Free University of Berlin, W. Germany
Muller, Kenneth, Carnegie Institute
Nelson, Margaret, Cornell University
Nicholls, John, Stanford University
Palka, John, University of Washington
Pearson, Keir, University of Alberta, Canada
Purpura, Keith, Columbia University
Quinn, William, Princeton University
Stewart, Randall, Texas Tech
Stewart, Walter, National Institutes of Health

Students[1]

Babbin, Catherine, Duke University
Beshers, Samuel, City College of New York
Chube, Ramona, Temple University
Consi, Thomas, Columbia University
Cottingham, Sandra, Indiana University
*DeRiemer, Susan, Yale University
Elias, Michael, Harvard University
Fritz, Jonathan, Washington University School of Medicine
*Gorczyca, Michael, Brandeis University

Gronenberg, Wulfila, Free University of Berlin, W. Germany
Itagaki, Haruhiko, Duke University
*Jelly, Karen, Wesleyan University
Kearns, Ruth, North Carolina State University
*Layton, Barry, McGill University, Montreal, Canada
Lebovitz, Richard, Johns Hopkins University
Lockery, Shawn, Yale University
*Miyamoto, Susan, University of Hawaii, Manoa
Phifer, Curtis, University of Kentucky
Plummer, Mark, Stanford University
Rankin, Mary Ann, University of Texas, Austin
Staley, Elizabeth, University of Connecticut
Tobias, Martha, Case Western Reserve University
Waldor, Matthew, Yale University
Wilde, Dixon, University of Rhode Island

Neurobiology

Instructors-in-chief

Hildebrand, John G., Columbia University
Reese, Thomas S., National Institutes of Health

Other faculty, staff, and lecturers

Battelle, Barbara A., National Institutes of Health (National Eye Institute)
Baughman, Robert, Harvard Medical School
Chun, Linda, Sidney Farber Cancer Institute
Crews, David, Harvard University
Diamond, Jack, McMaster University
Dowling, John E., Harvard University
Fambrough, Douglas, Carnegie Institution, Baltimore
Fischbach, Gerald D., Harvard Medical School
Fox, Thomas O., Childrens Hospital Medical Center, Boston
Frank, Eric, Harvard Medical School
Furshpan, Edwin J., Harvard Medical School
Goy, Michael, Harvard Medical School
Graham, William, National Institutes of Health
Hall, Linda M., Albert Einstein College of Medicine
Holtzman, Eric, Columbia University
Huttner, Susanne, University of California, Los Angeles
Kaneko, Akimichi, National Institute of Physiological Sciences, Japan
LaFratta, James, Harvard Medical School
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Landis, Story, Harvard Medical School
MacLeish, Peter, Harvard Medical School
Matsumoto, Steven G., Harvard Medical School
O'Lague, Paul, University of California, Los Angeles
Pastan, Ira, National Institutes of Health
Potter, Camilla, Swarthmore College
Potter, David D., Harvard Medical School
Rahamimoff, Rami, Hebrew University Medical School, Israel
Raviola, Elio, Harvard Medical School
Reese, Bonnie, National Institutes of Health
Schmidt-Glenewinkel, Thomas, Albert Einstein College of Medicine
Schneiderman, Anne, Harvard Medical School

Schroeder, Bryan, National Institutes of Health
Siegel, Ruth, Harvard Medical School
Truman, James W., University of Washington
Venkatesh, T. R., Albert Einstein College of Medicine
Zigmond, Richard E., Harvard Medical School

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*Hayashi, Jon, University of North Carolina
*Kayalar, Celik, Massachusetts Institute of Technology
*Kintner, Christopher, University of Wisconsin
*Lynch, Kathryn, Uniformed Services University of the Health Sciences
*McGuire, Barbara, University of Pennsylvania
*Nottebohm, Fernando, Rockefeller University
*O'Brien, Richard, Harvard Medical School
*Prillinger, Linde, Universität Regensburg, West Germany
*Rand, F. Peter, Brock University, Canada
*Tsui, Hoching Tiffany, Northwestern University

Physiology

Instructor-in-chief

Van Holde, Kensal E., Oregon State University

Other faculty, staff, and lecturers

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Axel, Richard, Columbia University
Ballinger, Dennis, Massachusetts Institute of Technology
Brown, Jay C., University of Virginia School of Medicine
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Cohen, William, Hunter College
Dentler, William, University of Kansas
Dreyfuss, Sideon, Massachusetts Institute of Technology
Elgin, Sarah, Harvard University
French, Cynthia, Massachusetts Institute of Technology
Gall, Joseph, Yale University
Graves, Theresa A., Harvard Medical School
Hamer, Dean, National Institutes of Health
Hamkalo, Barbara, University of California, Irvine
Hamlin, Joyce, University of Virginia
Hepler, Peter K., University of Massachusetts, Amherst
Hereford, Lynna, Brandeis University
Howe, Christine L., Yale University
Hunt, Tim, University of Cambridge, England, U. K.
Ide, Gregory S., Oregon State University
Inoué, Shinya, Marine Biological Laboratory/University of Pennsylvania
Jacobson, Bruce, University of Massachusetts, Amherst
Kaminer, Benjamin, Boston University School of Medicine
Kew, David, Wesleyan University
Klotz, Lynn, Princeton University
Kozak, Marilyn, University of Pittsburgh
Lerner, Michael, Yale University

Miller, Molly, Massachusetts Institute of Technology
Mooseker, Mark S., Yale University
Murray, Andrew, Harvard University
Pollard, Harvey, National Institutes of Health
Reeder, Ronald H., Fred Hutchinson Cancer Research Center
Roan, Judith G., Fred Hutchinson Cancer Research Center
Rosenbaum, Joel L., Yale University
Rubin, Terry, Sidney Farber Cancer Institute
Sheetz, Michael, University of Connecticut
Simpson, Robert, National Institutes of Health
Stephens, Raymond E., Boston University Medical School/Marine Biological Laboratory
Storrie, Brian, Virginia Technological Institute
Szent-Gyorgi, Andrew, Brandeis University
Varsavsky, Alex, Massachusetts Institute of Technology
Weber, Anne Marie, University of Pennsylvania

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*Aggeler, Judith, University of California, San Francisco
*Beckerle, Mary, University of Colorado
*Bennett, Holly, Albert Einstein College of Medicine
*Bonder, Edward, University of Pennsylvania
*Busa, William, University of California, Davis
*Cariello, Lucio, Stazione Zoologica di Napoli, Italy
*Cortadas, Jordi, Escuela Técnica Superior de Ingenieros Industriales, Spain
Cremo, Christine, Oregon State University
*Dunn-Coleman, Nigel, University of Virginia
*Eimstad, Robert, University of Massachusetts
French, Kathryn, Stanford University
*Hays, Thomas, University of North Carolina
*Herron, G. Scott, Oregon State University
*Landzberg, Michael, Columbia College
*L'Hernault, Steven, Yale University
*Lisak, Joan, Massachusetts Institute of Technology
*Love, Paul, University of Rochester School of Medicine
*McCarthy, Michael, Wesleyan University
Miller, Karen, Oregon State University
*Oliver, Patrice, University of Houston
Pearl, Mirilee, Cornell Medical College
*Porrello, Kathryn, University of California, Berkeley
*Render, Jo Ann, University of Texas, Austin
*Rieder, Vicki, Brandeis University
Rodi, Charles, University of Minnesota
*Ropson, Ira, Johns Hopkins University
*Sato, Masahiko, Dartmouth College
Shih, Cheng-Kon, Rockefeller University
*Smith, Francine, Johns Hopkins University
*Sosnowski, Ronald, Johns Hopkins University
*Stafstrom, Joel, University of Colorado
Stevens, Bryn, University of California, Los Angeles
Taylor, Walter, Harvard University
*Wadsworth, Patricia, Dartmouth College
*Wahlberg, Lars, University of Houston

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[1] All summer students listed completed the formal course programs. Asterisk indicates those completing post-course research sessions.

JANUARY

BEHAVIOR

Instructor-in-chief

ATEMA, JELLE, Boston University/Marine Biological Laboratory

Other faculty, staff, and lecturers

ALKON, DANIEL, National Institutes of Health/Marine Biological Laboratory
BARLOW, ROBERT, Syracuse University
BERG, CARL J., JR., Columbia University/Harvard University/Marine Biological Laboratory
CROW, TERRY, Marine Biological Laboratory
BRIDGES, ROBERT, Harvard Medical School
BRYANT, BRUCE, Boston University/Marine Biological Laboratory
BROWN, FRANK, JR., Woods Hole, Massachusetts
BRISBIN, I. LEHR, Savannah River Ecology Program
CALLARD, GLORIA, Harvard Medical School
CAREY, FRANCIS, Woods Hole Oceanographic Institution
CLARK, CHRIS, State University of New York, Stony Brook
DETHIER, VINCENT, University of Massachusetts, Amherst
ELGIN, RANDALL, Boston University/Marine Biological Laboratory
FRAZIER, JEAN, Brandeis University
JACOBSON, STUART, Sherborn, Massachusetts
KALMIJN, ADRIANUS, Woods Hole Oceanographic Institution
KAMIL, AL, University of Massachusetts, Amherst
KANWISHER, NANCY, Woods Hole, Massachusetts
KREITHEN, MEL, Cornell University
LEVIN, JOSEPH, Harvard University
MAYO, CHARLES, Center for Coastal Studies
MÜLLER-SCHWARZE, DIETLAND, State University of New York, Syracuse
PAYNE, KATY, Lincoln, Massachusetts
POLICANSKY, DAVID, University of Massachusetts, Boston
POLLACK, GERALD, Cornell University
RISTAU, CAROLYN, Rockefeller University
SMITH, DOUGLAS, Tams Engineers and Architects
STENZLER, DAN, East Setauket, New York
STUART, ALASTAIR, University of Massachusetts, Amherst
SULZMAN, FRANK, State University of New York, Binghamton
SWAIN, TONY, Boston University
TAMM, SIDNEY, Boston University Marine Program
WATKINS, WILLIAM, Woods Hole Oceanographic Institution
WILLIAMS, JANET, Swarthmore College
WILLIAMS, TIMOTHY, Swarthmore College

Students

BARSHAW, DIANA, Cornell University
BARTON, KAREN, Wells College
BELL, JANICE, University of South Carolina
CASEY, JOYCE, Hamilton College
DOUGLASS, JOHN, Oberlin College
KOCH, MICHAEL, Gustavus Adolphus College
LAVELLI, KARI LEE, Wells College
MOFFETT, MARK, Beloit College
MOORE, KAREN, Woods Hole Oceanographic Institution
MULCAHY, RICHARD, Colby College
ROSENBLUTH, BETSY, University of Oregon

SMITH, BARBARA, Northeastern University
ULBRICH, ROBERT, Hamilton College
WAY, CATHY ANN, University of Texas School of Public Health

COMPARATIVE PATHOLOGY OF MARINE INVERTEBRATES

Instructor-in-chief

BANG, FREDERIK B., Johns Hopkins University

Other faculty, staff, and lecturers

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EDDS, KENNETH T., State University of New York, Buffalo
FARLEY, C. AUSTIN, NOAA Marine Fisheries Laboratory, Oxford, Maryland
JOHNSON, PHYLLIS T., NOAA Marine Fisheries Laboratory, Oxford, Maryland
LEVIN, JACK, Johns Hopkins University
MAJNO, G., University of Massachusetts, Worcester
PAN, S., Harvard University
PRENDERGAST, ROBERT A., Johns Hopkins University
REINISCH, CAROL L., Harvard University
SILVERSTEIN, ARTHUR, Wiloner Clinic, Johns Hopkins Hospital
WOLKE, R., University of Rhode Island

Students

BELL, KATHRYN, McGill University, Canada
BELTON, CAROL, Johns Hopkins University
CAMPBELL, DAVID, Johns Hopkins University
COX, CATHERINE, State University of New York, Downstate Medical Center
ERIKSON, MARK, Wistar Institute
FOSTER, CAROLYN, University of Washington
RITTENBURG, JAMES, University of Maine
SHORT, SANDRA, University of Delaware
SPITSBERGEN, JAN, Michigan State University College of Veterinary Medicine
WANG, NISSI S., Johns Hopkins University

DEVELOPMENTAL BIOLOGY

Instructor-in-chief

VINCENT, WALTER S., University of Delaware

Other faculty, staff, and lecturers

BELL, EUGENE, Massachusetts Institute of Technology
COLLIER, JACK, Brooklyn College
EDDS, KENNETH, State University of New York, Buffalo
GERBI, SUSAN, Brown University
GRASSLE, JUDITH, Marine Biological Laboratory
GROSS, PAUL, Marine Biological Laboratory
INOUÉ, SHINYA, Marine Biological Laboratory
LASH, JAY, University of Pennsylvania
LAUFER, HANS, University of Connecticut
MARCUS, NANCY, Woods Hole Oceanographic Institution
MASER, MORTON, Marine Biological Laboratory
MILLER, RICHARD, Temple University
PAGE, JENNIFER, Tulane University
RUDERMAN, JOAN, Harvard University

STARKE, LYNN, Wheaton College
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TASCA, RICHARD, University of Delaware
TILNEY, LEWIS, Marine Biological Laboratory/University of Pennsylvania

Students

BARNETT, MARCUS, Morehouse College
BREGG, BARBARA, College of St. Rose
COLLINS, JAN, Eisenhower College
CORBETT, FRANCES, Texas Southern University
CORMIER, ETHEL, Texas Southern University
DELEHANTY, ANN, Nasson College
HOUSTON, WILLIE, JR., Kentucky State University
KELNER, KATRINA, Baylor College of Medicine, Texas Medical Center
MORRIS, ABRAHAM, Tougaloo College
MURRAY, CAROLYN, Morgan State University
PREDIGER, ELLEN, Macalester College
SCALISE, FREDERICK, Westat Research Corporation
WESOLOWSKI, MARY, Clark University

ECOLOGY

Instructors-in-chief

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HOBBIE, JOHN E., Marine Biological Laboratory

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BILLINGS, W. DWIGHT, Duke University
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BURROUGHS, RICHARD H., Marine Biological Laboratory
DEWAR, ROBERT, University of Connecticut
EMERY, KENNETH O., Woods Hole Oceanographic Institution
FOY, DOUGLAS, Conservation Law Foundation
GRASSLE, J. FREDERICK, Woods Hole Oceanographic Institution
HAY, KEITH, American Petroleum Institute
HENNEMUTH, RICHARD, National Marine Fisheries Service Laboratory
HOUGHTON, RICHARD A., Marine Biological Laboratory
HOWARTH, ROBERT W., Marine Biological Laboratory
LIKENS, GENE E., Cornell University
LOVEJOY, THOMAS E., World Wildlife Fund
MELILLO, JERRY M., Marine Biological Laboratory
MOORE, BERRIEN, University of New Hampshire
MORRIS, JAMES M., Marine Biological Laboratory
NAIMAN, ROBERT J., Woods Hole Oceanographic Institution
PETERSON, BRUCE J., Marine Biological Laboratory
REMINGTON, CHARLES L., Yale University
RICHARDS, ALISON, Yale University
SANDERS, HOWARD L., Woods Hole Oceanographic Institution
SCHNEIDER, ERIC, University of Rhode Island
SHAVER, GAIUS R., Marine Biological Laboratory
SMITH, FREDERICK E., Harvard University
STROTHER, PAUL, Harvard University
TEAL, JOHN M., Woods Hole Oceanographic Institution

Students

Bernard, David, LaGrange, Illinois
Bossort, Kathleen, University of Calgary, Canada
Cox, Wendy, Niagara Falls, Canada
Donlon, Brian, University of Delaware
Eifert, Robert, Stetson University
Fagan, Ann Marie, Cornell University
Ford, William, Morgan State University
Hannibal, Charles, Texas Southern University
d'Hedouville, Joan, Canton School System
Heyn, Michael, Murray State University
Lee, Let, National Park Services
Marston, Blythe, San Francisco, California
Pinkney, Alfred, Clement Associates, Inc.
Prosper, Truit, Dillard University
Schmitt, Barbara Jo, University of Wisconsin, Madison
Strahm, Wendy, Oberlin College
Tanner, Carol, Tougaloo College
Vallas, Donna, Williams College

NEUROBIOLOGY

Instructor-in-chief

Alkon, Daniel L., National Institutes of Health/Marine Biological Laboratory

Other faculty, staff, and lecturers

Adelman, William J., Jr., National Institutes of Health/Marine Biological Laboratory
Atwood, Harold L., University of Toronto
Barlow, Robert B., Sr. Syracuse University
Brightman, Milton W., National Institutes of Health
Connor, John A., University of Illinois
Corson, D. Wesley, Marine Biological Laboratory
Crow, Terry J., National Institutes of Health/Marine Biological Laboratory
DeFelice, Louis J., Emory University School of Medicine
Dowling, John E., Harvard University
Fritz, Lawrence, Rockefeller University
Furshpan, Edwin J., Harvard Medical School
Govind, C. K., University of Toronto/Marine Biological Laboratory
Harosi, Ferenc, Marine Biological Laboratory/Boston University
Jacklet, Jon W., State University of New York, Albany
Kaplan, Ehud, Rockefeller University
Kravitz, Edward A., Harvard Medical School
Kuzirian, Alan M., National Institutes of Health/Marine Biological Laboratory
Lederhendler, I. Izja, National Institutes of Health/Marine Biological Laboratory
MacNichol, Edward F., Marine Biological Laboratory/Boston University
Mauro, Alexander, Rockefeller University
Neary, Joseph T., National Institutes of Health/Marine Biological Laboratory
Price, Christopher H., Boston University
Rall, Wilfrid, National Institutes of Health
Raymond, Stephen A., Massachusetts Institute of Technology
Shepherd, Gordon M., Yale University School of Medicine
Shoukimas, Jon J., National Institutes of Health/Marine Biological Laboratory
Szuts, Ete Z., Marine Biological Laboratory
Wiesel, Torsten N., Harvard Medical School

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Keating, Arthur, Jr., University of New Hampshire
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Lancet, Doron, Harvard University
Lipshultz, Richard, University of Rochester
Naden, Charles, Quinnipiac College
Nelson, Chauncy, Dillard University
Newman, Janet, Oberlin College
Plancher, Kevin, Trinity College
Richards, William, Tufts University
Rittenhouse, Ann, Boston University
Rosengard, Bruce, Tufts University
Solish, Sharyn, State University of New York, Downstate Medical Center
Stevenson, Rodrick, Dillard University
Udovc, Judith, University of Wisconsin, Madison

SHORT COURSES

Biological Electron Microscopy for Technicians

Instructors-in-chief

Maser, Morton D, Marine Biological Laboratory
Scarborough, Ann, Johns Hopkins University

Other faculty, staff, and lecturers

Copeland, D. Eugene, Marine Biological Laboratory
Houghton, Susan, Woods Hole Oceanographic Institution
Marquis, Sally, Marine Biological Laboratory
Waterbury, John, Woods Hole Oceanographic Institution

Students

Adler, Catherine, St. Vincent Charity Hospital
Anderson, Kathy, Syracuse University
Auerbach, L. Philip, Lafayette College
de Avendano, Yolanda, University of Ottawa, Canada
Eisner, Maria, Cornell University
Geiser, Albert L., Hahnemann Medical College and Hospital
Isenberg, George R., State University of New York, Potsdam
Kmetz, Marjorie E., Rutgers Medical School
McCall, Carol, University of Wisconsin
Ploetz, Karin, William Paterson College
Rostand, Katherine S., University of Alabama Medical Center
Seiden, Leslie, Cornell University Medical College

Analytical and Quantitative Light Microscopy in Biology, Medicine and Materials Science

Instructor-in-chief

Inoué, Shinya, Marine Biological Laboratory/University of Pennsylvania

Other faculty, staff, and lecturers

Brenner, Mel, Nikon, Inc.
Chaisson, Richard, Olympus Corporation of America
Cohen, David, Venus Scientific, Inc.
Ellis, Gordon, University of Pennsylvania
Harosi, Ferenc, Marine Biological Laboratory
Houghton, Susan, Woods Hole Oceanographic Institution
Kara, G. F., Carl Zeiss, Inc.
Keller, Ernst, Carl Zeiss, Inc.
Levy, Stuart, E. Leitz, Inc.
Lutz, Douglas, University of Manitoba, Canada
Marquis, Sally, Marine Biological Laboratory
McManus, Jerry, E. Leitz, Inc.
Salmon, Edward D., University of North Carolina
Sawyer, William, Carl Zeiss, Inc.
Taylor, D. Lancing, Harvard University
Taylor, Richard, Colorado Video
Woodward, Bertha, Marine Biological Laboratory
Zeh, Robert, State University of New York, Albany
Perrin, Don, Crimson Camera Technical Sales, Inc.

Students

Chen, Victor, State University of New York, Buffalo
Frantz, Catherine, University of Chicago
Garland, Carol, American Cyanamid
Huebner, Erwin, Marine Biological Laboratory
Sims, David, Boone County Hospital
Kronebusch, Paul, University of Wisconsin
McCann, Mary, Polaroid Corporation
Saunders, Mary Jane, University of Massachusetts
Silver, Robert, University of California, Berkeley
Solomon, Dennis, Newton Centre, Massachusetts
Thaler, Davis, University of Massachusetts
Verma, Vinod, National Institutes of Health
Woodrum, Diane, Harvard Medical School

Freeze-Etching in Electron Microscopy

Instructor-in-chief

Steere, Russell L., U. S. Department of Agriculture

Other faculty, staff, and lecturers

Erbe, Eric, U. S. Department of Agriculture
Graham, William, National Institutes of Health
Houghton, Susan, Woods Hole Oceanographic Institution
Marquis, Sally, Marine Biological Laboratory
Rash, John E., Colorado State University
Sommer, Joachim R., Duke University Medical Center

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Braun, Susan Jean, Veterans Administration Medical Center, Albany
Buchanan, Glenn, Armed Forces Radiobiology Research Institute
Burton, Jarrett L., National Red Cross
Holtzman, Eric, Columbia University

MAKUCHAN, EILEEN, University of Hawaii
NORDEN, JEANETTE, Vanderbilt University School of Medicine
OLSON, MARK, University of North Dakota School of Medicine
PIEKOS, BARRY, Yale University
RAMBERG, JANE, Oklahoma State University
SILVERMAN, HAROLD, University of Toronto
STUDHOLME, KEITH, Hofstra University
TOOR, ELIZABETH, Carnegie-Mellon University
TROUTH, C. OVID, Howard University College of Medicine
WELDON, PETER, McGill University, Canada

ELECTRON MICROSCOPY IN THE BIOLOGICAL SCIENCES

Instructors-in-chief

BOWERS, BLAIR, National Institutes of Health
MASER, MORTON D, Marine Biological Laboratory

Other faculty, staff, and lecturers

HOUGHTON, SUSAN, Woods Hole Oceanographic Institution
MARQUIS, SALLY, Marine Biological Laboratory
PEACHEY, LEE D., University of Pennsylvania
PORTER, KEITH R., University of Colorado
SCARBOROUGH, ANN, Johns Hopkins University
WATERBURY, JOHN, Woods Hole Oceanographic Institution
WILLINGHAM, MARK C., National Institutes of Health

Students

ACEVEDO, RAUL, University of Puerto Rico
CARLEY, WAYNE W., College of the Holy Cross
COULTER, JOE DAN, University of Texas Medical Branch
DAUGHERTY, CYNTHIA, Children's Hospital Medical Center, Cincinnati
GLADE, RICHARD W., University of Vermont
LEFLORE, WILLIAM B., Spelman College
MASSARI, V. JOHN, Howard University College of Medicine
MCCORMICK, JOHN F., Montefiore Hospital and Medical Center
PATTERSON, ROSALYN, Atlanta University
QUARONI, ANDREA, Massachusetts General Hospital
SPIELHOLZ, NEIL, New York University Medical Center
TUCKER, ROBERT W., Johns Hopkins University

SMALL COMPUTERS IN BIOMEDICAL RESEARCH, I

Instructor-in-chief

PALMER, LARRY, University of Pennsylvania

Other faculty, staff, and lecturers

MARQUIS, SALLY, Marine Biological Laboratory
PEACHEY, LEE D., University of Pennsylvania

Students

BRINKLEY, LINDA L., University of Michigan
CAMPBELL, DAVID, Emory University Clinic
DE WEER, PAUL, Washington University School of Medicine
EDMONDS, HARVEY L., JR., University of Louisville
GOLDSTEIN, MARGARET ANN, Baylor College of Medicine

GUTHRIE, GEORGE D., Indiana University School of Medicine
HAHIN, RICHARD, State University of New York, Stony Brook
HOLTZMAN, ERIC, Columbia University
KENNEDY, WILLIAM R., University of Minnesota
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Rose, Birgit, University of Miami
Rosenkranz, Prina, Barnard College, Columbia University
Rosenthal, Eric, Harvard Medical School
Rushbrook, Julie, State University of New York, Downstate Medical Center
Saito, Takehiko, St. Marianna Medical School, Japan
Sans, Alain, Université de Montpellier II, France
Saunders, Barry F., University of North Carolina, Chapel Hill

Scott, James, University of Florida College of Medicine
Scruggs, Virginia, University of Miami
Sejnowski, Terrence J., Harvard Medical School
Serhan, Charles M., New York University Medical Center
Shoaf, Sarah A., Johns Hopkins University
Shoup, Thomas L., Emory University
Shure, Michael, Yale University
Simon, Bruce, University of Iowa
Simon, Sanford, New York University Medical Center
Smiley, Kathleen A., Worcester Foundation for Experimental Biology
Smith, Gilbert H., Washington University
Smith, Laurens, Jr., University of Maryland
Smith, Patricia J., State University of New York, Purchase
Socci, Robin Richard, Rutgers University
Spivack, Warren D., Institute for Basic Research in Mental Retardation
Spray, David C., Albert Einstein College of Medicine
Stathoplos, Linda, Swarthmore College
Steele, Joy, University of Alberta, Canada
Steele, Roderick, National Institutes of Health
Steinacker, Antoinette, Rockefeller University
Stern, Jeffrey, Brandeis University
Sternlicht, Himan, Case Western Reserve University
Stich, Thomas, University of Maryland
Stokes, Darryl, Emory University
Sugimori, Mutsuyuki, New York University Medical Center
Suprenant, Kathy Ann, University of Virginia
Susan, Stanley R., Kresge Eye Institute, Wayne State University
Swenson, Randolphe P., University of Pennsylvania
Szaro, Ben, Johns Hopkins University
Szent-Kirolyi-Szent-Gyorgyi, Eva M., Brandeis University
Tauck, David L., Duke University
Taylor, Stuart, Mayo Clinic
Thomas, Roger C., Yale University
Tiffert, Teresa, University of Maryland School of Medicine
Timpe, Leslie C., Harvard Medical School
Tompkins, Robert, Tulane University
Tsai, T. D., Baylor College of Medicine
Tytell, Michael, Case Western Reserve University
Vander Zalm, Johan M., Vakgroep Veterinaire Farmakologie en Toxicologie, The Netherlands
Veenstra, Richard, University of Iowa
Viverberg, Henk P. M., Institut voor Veterinaire Farmakologie en Toxical, The Netherlands
Wahl, Michael, Case Western Reserve University
Walrond, John, National Institutes of Health
Wang, Howard, University of California, Santa Cruz
Warren, Mary K., University of Maryland
Weiss, Jerry, Northwestern University Medical Center
Wells, Jay B., National Institutes of Health
West, Hillary, Illinois State University
Westerfield, Richard M., Harvard Medical School
Whitehorn, Erik A., Wistar Institute
Wolniak, Stephen M., University of Massachusetts, Amherst
Wood, Anne Michelle, University of Chicago
Worgul, Basil, Columbia University
Worthington, Alma, Carnegie Mellon University

Wu, Chau H., Northwestern University Medical School
Wu, Shang-Chin, Institute of Oceanography, People's Republic of China
Yeh, Jay Z., Northwestern University
Yen, Lung-Fei, Peking Agricultural University, People's Republic of China
Yulo, Teresa, University of Rochester Medical Center
Zackroff, Robert V., Carnegie Mellon University
Zakevisius, Jane M., New York University School of Medicine
Zimering, Mark B., Albert Einstein College of Medicine

YEAR-ROUND PROGRAMS

Boston University Marine Program (BUMP)

Director

Humes, Arthur G., Boston University

Staff

Allen, Sarah, Boston University
Atema, Jelle, Boston University
Barshaw, Diana, Boston University
Beale, Eleanor, Boston University
Cogswell, Charlotte, Boston University
Costello, Walter, Yale University
Cushman, Mary, Boston University
Elgin, Randall, Boston University
Gibson, Daniel, University of Texas
Govind, C. K., University of Toronto, Canada
Hahn, Dorothy, Boston University
Harrington, Robin, Boston University
Hartman, Jean, Boston University
Hill, Lena, Boston University
Karnofsky, Lisa, Boston University
Leavitt, Dale, Boston University
Meiss, Dennis, Clark University
Oleszki-Szuts, Susan, Boston University
Pearce, Joanna, Boston University
Price, Christopher, Boston University
Rietsma, Carol, State University of New York, New Paltz
Tamm, Sidney, Boston University
Tamm, Signhild, Boston University
Trinkaus-Randall, Vickery, Boston University
Valiela, Ivan, Boston University
Van Etten, Richard, Boston University
Volkmann, Suzanne, Boston University
Yamin, Michael, Boston University

Trainees (of Boston University unless otherwise noted)

Anderson, David M., Tufts University
Bauer, James
Borelli, John
Boroni, Paola Ferma
Botero, Leonor
Bryant, Bruce
Bryant, Donald
Buchsbaum, Robert
Caraco, Nina
David, Cabell
Derby, Charles
DeRubeis, Robert
Divins, David
Dojiri, Masahiro
Donohue, Melanie
Dourdeville, Theodore

Duncan, Thomas
Faucher, Bruce
Foreman, Kenneth
Fujita, Rodney
Giblin, Anne
Hill, Russell
Hook, Jamie
Howes, Brian
Maciolek, Nancy
McCall, Claire
Miller, Carl
Mills, Susan Wier
Moss, Anthony
Pascoe, Natalie
Poole, Alan
Trott, Thomas
Webb, Jacqueline
Whiteside, Lisa
Williams, Isabelle
Wilson, John

Developmental and Reproductive Biology Laboratory

Director

Gross, Paul R., Marine Biological Laboratory

Staff

O'Loughlin, John, Marine Biological Laboratory
Simpson, Robert T., National Institutes of Health

The Ecosystems Center

Director

George M. Woodwell, Marine Biological Laboratory

Staff and consultants (all of Marine Biological Laboratory)

Banner, Steven
Boone, Richard D.
Bowles, Francis P.
Burroughs, Richard H. (on leave 1980)
Carlson, Christopher
Chan, Yip-Hoi
Connor, Michael
Corliss, Teresa A. L.
Eldred, Kate
Elkin, Kerry M.
Eshleman, Keith N.
Friedlander, Amy I.
Garritt, Robert W.
Gibbs, Richard K.
Helfrich, John V. K.
Hobbie, John E.
Howarth, Robert W.
Houghton, Richard A.
Jordan, Marilyn B.
Juers, David W.
Kane, Ann E.
Kijowski, Voytek
Lajtha, Kathryn A.
Lipschultz, Frederic
Luchessa, Karen J.
Marinucci, Andrew C.
Marquis, Sally L.
Melillo, Jerry M.
Mlodzinska, Kasia
Montgomery, Mary Louise
Morris, James T.
Palm, Cheryl A.
Parsons, Katherine C.
Peterson, Bruce J.
Petrecca, Rosemarie F.
Pilling, Susan E.
Schimel, Joshua
Semino, Suzanne J.
Sladovich, Hedy E.
Steudler, Paul A.
Turner, Andrea R.
Upton, Joan M.
Zacks, Charles

Trainees

Ammons, J. Timothy, Intern
Bowden, William B., University of North Carolina, Year-in-Science
Dick, Randall W., Intern

Fownes, James N., Intern
Gale, Judith, Intern
Hoffman, Julie, University of California, Santa Cruz, Year-in-Science
Kriet, Keith, Yale University, Year-in-Science
Lang, Helen E., Intern
Morse, Julie K., Intern
Mullin, Elizabeth D., Yale University, Year-in-Science
Reed, James P., University of North Carolina, Year-in-Science
Wargo, John P., Yale University, Year-in-Science
Warren, James, Yale University, Year-in-Science

Laboratory of Biophysics

Director

Adelman, William J., Jr., NINCDS-NIH

Staff

Acosta-Urquidi, Juan, NINCDS-NIH
Alkon, Daniel L., NINCDS-NIH
Attardo, Linda S., Wheaton College
Barnes, Edward, NINCDS-NIH
Belamarich, Peter F., Boston University Medical School
Buchanan, JoAnn, Northeastern University
Crow, Terry J., NINCDS-NIH
DeFelice, Louis J., Emory University
Fohlmeister, Jurgen F., University of Minnesota
Goh, Yasumasa, NINCDS-NIH
Goldman, David E., State University of New York at Binghamton
Harrigan, June F., NINCDS-NIH
Hill, Lena, NINCDS-NIH
Hodge, Alan J., NINCDS-NIH
Kuzirian, Alan, NINCDS-NIH
Kuzirian, Jeanne, NINCDS-NIH
Lederhendler, Izja, NINCDS-NIH
Leighton, Stephen, NINCDS-NIH
Leonard, Dorothy A., NINCDS-NIH
Mueller, Ruthanne, NINCDS-NIH
Neary, Joseph T., NINCDS-NIH
Olds, James, University of California, Irvine
Richards, William, Tufts University
Ryan, Liane E., NINCDS-NIH
Senft, Stephen L., University of Oregon
Shiman, Leon G., NINCDS-NIH
Shoukimas, Jonathan J., NINCDS-NIH
Takeda, Toshiaki, NINCDS-NIH
Tyndale, Clyde, NINCDS-NIH
Waltz, Richard, NINCDS-NIH
Wells, Jay B., NINCDS-NIH
West, Alexander, NINCDS-NIH

Laboratory of Sensory Physiology

Director

MacNichol, Edward F., Jr., Marine Biological Laboratory

Staff

BOURGOIN, FLORENCE L., Marine Biological Laboratory
COLLINS, BARBARA ANN, Marine Biological Laboratory
COOK, PATRICIA, Marine Biological Laboratory
CORSON, D. WESLEY, Marine Biological Laboratory
FEIN, ALAN, Marine Biological Laboratory
GOODMAN, STEVEN, Marine Biological Laboratory
HAROSI, FERENC I., Marine Biological Laboratory
LEVINE, JOSEPH S., Marine Biological Laboratory
LEVY, SIMON, Marine Biological Laboratory
SZUTS, ETE ZOLTAN, Marine Biological Laboratory

NATIONAL FOUNDATION FOR CANCER RESEARCH

Director

SZENT-GYÖRGYI, ALBERT, Marine Biological Laboratory

Staff

GASCOYNE, PETER R. C., Marine Biological Laboratory
LEWIS, T. JOHN, University College of North Wales, United Kingdom
MCLAUGHLIN, JANE A., Marine Biological Laboratory
MEANY, RICHARD A., Marine Biological Laboratory
PETHIG, RONALD, University College of North Wales, United Kingdom

LABORATORY OF D. EUGENE COPELAND

Director

COPELAND, D. EUGENE, Marine Biological Laboratory

LABORATORY OF JUDITH P. GRASSLE

Director

GRASSLE, JUDITH P., Marine Biological Laboratory

Staff

BIRTWISTLE, FERN, Marine Biological Laboratory
HOLBROOK, JANE, Dalhousie University
MILLS, SUSAN, Marine Biological Laboratory
PHILBIN-MUNSON, MAYBELLINE, Marine Biological Laboratory

LABORATORY OF SHINYA INOUÉ

Director

INOUÉ, SHINYA, University of Pennsylvania/Marine Biological Laboratory

Staff

EISEN, ANDREW, University of Pennsylvania
GORDON, JERRY, Marine Biological Laboratory
HORN, ED, University of Pennsylvania
INOUÉ, CHRISTOPHER, Marine Biological Laboratory
INOUÉ, THEODORE, Marine Biological Laboratory
LUTZ, DOUGLAS, University of Pennsylvania
WOODWARD, BERTHA, University of Pennsylvania

Visiting/Collaboratory Investigators

Burgos, Mario, Harvard Medical School
Cohen, David, Venus Scientific, Inc.
Dan, Katsuma, Tokyo Metropolitan University, Japan
Ellis, Gordon, University of Pennsylvania
Fujiwara, Keigi, Harvard Medical School
Okazaki, Kayo, Tokyo Metropolitan University, Japan
Tilney, Lewis, University of Pennsylvania
Woodruff, Richard, West Chester State College

Laboratory of Eric Kandel

Director

Kandel, Eric, Columbia University

Staff

Berg, Carl J., Jr., Columbia University/Harvard University/Marine Biological Laboratory
Capo, Thomas, Columbia University
Paige, John A., Columbia University
Perritt, Susan, Columbia University

Laboratory of Jeffry B. Mitton

Director

Mitton, Jeffry B., University of Colorado

Laboratory of Raymond E. Stephens

Director

Stephens, Raymond E., Boston University Medical School/Marine Biological Laboratory

Staff

Porter, Mary E., Marine Biological Laboratory/University of Pennsylvania
Pratt, Melanie, Harvard Medical School
Stommel, Elijah, Boston University Medical School/Marine Biological Laboratory
Suprenant, Kathy, University of Virginia

Laboratory of Lewis G. Tilney

Director

Tilney, Lewis G., University of Pennsylvania

Laboratory of Ruth D. Turner

Director

Turner, Ruth D., Harvard University

Staff

Berg, Carl J., Jr., Columbia University/Harvard University/Marine Biological Laboratory
King, Darcy, Marine Biological Laboratory
Tracey, Gregory A., Harvard University

XI. HONORS

FRIDAY EVENING LECTURES

BROWN, FRANK A., JR., Marine Biological Laboratory, January 11 "*An Exogenous Contribution to Biological Clocks*"

LIKENS, GENE E., Cornell University, January 18 "*Acid Rain: Causes to Consequences*"

WIESEL, TORSTEN N., Harvard University Medical School, January 25 "*Functional Architecture of the Mammalian Visual Cortex*"

PORTER, KEITH R., University of Colorado, June 27 "*Some Observations on the Cytoplasmic Ground Substance (Fundamental Substance)*"

BOGORAD, LAWRENCE, Harvard University, July 4 "*Is it Independence Day for Chloroplasts, Too?*"

BEAN, CHARLES, General Electric Corporation, July 11 "*How the Sperm Find the Egg*"

KATZ, SIR BERNARD, University College, London, July 17, 18, Forbes Lectures. "*Synaptic Transmission: from Cellular to Molecular Action. I. The Subcellular Process of Transmitter Release. II. Elementary Actions at the Postsynaptic Membrane*"

STRUMWASSER, FELIX, California Institute of Technology, July 25 Lang Lecture. "*Peptidergic Neurons, Neuroactive Peptides, and Circadian Oscillators Controlling Behavior in* Aplysia"

BALTIMORE, DAVID, Massachusetts Institute of Technology, August 1 "*Decision-Making in the Immune System*"

RAFF, RUDOLF, Indiana University, August 8 Zwilling Lecture. "*Organization of the Genes for Tubulin in* Drosophila, *and their Expression in Development*"

CRISTOFALO, VINCENT J., Wistar Institute. August 15 "*Cellular Senescence: Factors Modulating Cell Proliferation* in vitro"

GELFANT, SEYMOUR, Medical College of Georgia, August 22 "*Cell Cycle Aspects of Tissue Aging, Immunosenescence, Psoriasis, and Tumor Growth*"

PORTER, RODNEY R., Department of Biochemistry, University of Oxford, England, August 29 "*Molecular Basis of Immunity to Infection*"

CHARLES A. LINDBERGH LECTURES IN ECOLOGY

HOLLING, C. S., University of British Columbia, July 16 "*Adapting to Uncertainty in an Unforgiving Society*"

EHRLICH, PAUL R., Stanford University, July 30 "*Should We Be Worried About the Extinction of Other Species?*"

GRASS FOUNDATION FELLOWS

ADAMS, DAVID J., University of Washington
BODZNICK, DAVID, Wesleyan University
BULLOCK, JAMES O., Rush-Presbyterian-St. Luke's Medical Center
COLLINS, STEPHEN D., Case Western Reserve University
DRAKE, PETER, Bryn Mawr College
EHRLICH, BARBARA E., University of California at Los Angeles
HARARY, HOWARD H., State University of New York at Stony Brook
KASS, LEONARD J., Eye Research Institute of the Retina Foundation
KRACKE, GEORGE R., Washington University School of Medicine
MELLOW, ALAN W., Northwestern University
REINGOLD, STEPHEN, Princeton University, Associate Program Director
RUEBEN, JOHN P., Columbia University, Program Director
RUBIN, LEONA J., University of Colorado Medical Center
SAIDEL, WILLIAM M., University of California at La Jolla
SANCHEZ, JORGE, Centro de Investigaciones del IPN, Mexico
STEPHENS, PHILIP, University of Toronto, Canada

JOSIAH MACY, JR., FOUNDATION FELLOWS AND STUDENTS

BROWN, MARY M., Knoxville College
CHUBE, RAMONA, Temple University
HOGAN, JAMES JR., University of Connecticut
HOWARD, GAIL, Morehouse College
JONES, RENA T., Spelman College
LEFLORE, WILLIAM, Spelman College
MEHROTRA, BHARATI, Tougaloo College
OLIVER, PATRICE, University of Houston
PATTERSON, ROSALYN, Atlanta University
PORTER, CHARLES, San Jose State University
SMITH, GEORGE W., Union College

STEPS TOWARD INDEPENDENCE FELLOWS

BEGG, DAVID, Harvard Medical School
BELL, WAYNE, Hamilton College
BOYER, BARBARA, Union College
BRENCHLEY, GAYLE, University of California at Irvine
BUSS, LEO, Yale University
CHARLTON, MILTON, Ohio University College of Medicine
FAMIGLIETTI, EDWARD JR., Wayne State University School of Medicine
GRINVALD, AMIRAM, Weizmann Institute of Science, Israel
MAGLOTT, DONNA, Howard University
SALAMA, GUY, University of Pennsylvania
SCHWAB, WALTER, Virginia Polytechnic Institute and State University
SHIRAI, HIROKO, National Institute for Basic Biology, Japan
SMITH, STEPHEN, University of California at Berkeley
TELZER, BRUCE, Pomona College
TREISTMAN, STEVEN, Bryn Mawr College

FATHER ARSENIUS BOYER FELLOWSHIPS

DILL, THERESE, University of Maryland
MOLLURA, FRANCESCA, LeMoyne College

GARY N. CALKINS MEMORIAL SCHOLARSHIP

MENDEL, EILEEN, Wesleyan University

FRANCES S. CLAFF MEMORIAL SCHOLARSHIP

DUNN-COLEMAN, NIGEL, University of Virginia

EDWIN GRANT CONKLIN MEMORIAL SCHOLARSHIP

MONK, PETER, Rutgers University

LUCRETIA CROCKER SCHOLARSHIPS

BEDARD, ANDRE, McGill University, Canada
BRANDL, HELMUT, Universitat Zurich, Switzerland
CAMPBELL, LISA, State University of New York, Stony Brook
CHIN, EDWARD JR., University of Georgia
CLANCY, TIMOTHY, University of Vermont

Coleman, James, North Carolina State University
Cortadas, Jordi, Escuela Tecnica Superior de Ingenieros Industriales, Spain
Crill, Patrick, University of North Carolina
Fitzgerald, Kathleen, University of Connecticut
Lessard, Evelyn, University of Rhode Island
Terracciano, Joseph, University of Massachusetts, Amherst
Wahlberg, Lars, University of Houston
Weber, Frederick, Northeastern University

Founders Scholarships

In 1980, these Scholarships were given in memory of:

W. C. Curtis
Caswell Grave
J. M. Johlin
S. O. Mast
A. P. Mathews
T. H. Morgan
A. H. Sturtevant

Recipients:

Bennett, Virginia, University of California, Santa Cruz
Cariello, Lucio, Stazione Zoologica di Napoli, Italy
Chambers, Randolph, University of Massachusetts
Cortadas, Jordi, Escuela Tecnica Superior de Ingenieros Industriales, Spain
DeSantis, Rosaria, Stazione Zoologica di Napoli, Italy
Dise, Nancy, University of Notre Dame
Hays, Thomas, University of North Carolina
Horgan, Erich, Hartwick College
Landzberg, Michael, Columbia University
Leighty, Bruce, Dartmouth College
Mansour, Randa, University of North Carolina
Thomson, James, University of Illinois, Champaign-Urbana
Wahlberg, Lars, University of Houston

Society of General Physiologists Scholars

Beckerle, Mary, University of Colorado
Pearl, Mirilee, Cornell Medical College

Frank R. Lillie Fellowships

Dan, Katsuma, Tokyo Metropolitan University, Japan
Monroy, Alberto, Stazione Zoologica di Napoli, Italy

Herbert W. Rand Fellowship

Kaneko, Akimichi, National Institute for Physiological Sciences, Japan

MBL Award for the Most Outstanding Paper Given at the MBL General Scientific Meetings of August 1979

"*A General Method, Employing Arsenazo III in Liposomes, for the Study of Calcium Ionophores: Results with A23187 and Prostaglandins.*"

By Charles Serhan, Paul Anderson, Elizabeth Goodman, Elisabet Samuelsson, and Gerald Weissmann of the New York University School of Medicine

XII. INSTITUTIONS REPRESENTED

U. S. A.

Academy of Natural Sciences of Philadelphia
Alabama, University of, Birmingham
Alabama, University of, Medical Center
Albert Einstein College of Medicine
American Cynamid
American Museum of Natural History
American Optical Corporation
American Petroleum Institute
Amherst College
Arizona, University of
Armed Forces Radiobiology Research Institution
Atlanta University
Auburn University

Ball State University
Barnard College
Batelle's William F. Clapp Laboratories
Baylor College of Medicine
Beloit College
Boone County Hospital
Boston Biomedical Research Institute
Boston University
Boston University School of Medicine
Brandeis University
Bridgeport, University of
Brock University
Brown University
Bryn Mawr College

California Institute of Technology
California, University of, Berkeley
California, University of, Davis
California, University of, Irvine
California, University of, La Jolla
California, University of, Los Angeles
California, University of, Riverside
California, University of, San Diego
California, University of, San Francisco
California, University of, Santa Barbara
California, University of, Santa Cruz
Carl Zeiss Inc.
Carnegie Institution of Baltimore
Carnegie Institution of Washington
Carnegie-Mellon University
Case Western Reserve University
Case Western Reserve University School of Medicine
Center for Coastal Studies
Chicago, University of
Children's Hospital Medical Center, Boston
Children's Hospital Medical Center, Cincinnati
CIBA-GEIGY Corporation
Cincinnati, University of
Cincinnati, University of, School of Medicine
Clark University
Colby College
Colorado, University of
Colorado, University of, Medical School
Colorado State University
Colorado Video
Columbia College
Columbia University
Columbia University, College of Physicians and Surgeons
Connecticut, University of
Connecticut, University of, Health Center
Connecticut, University of, Medical School
Conservation Law Foundation
Cornell Medical College
Cornell University
Creighton University Medical School
Crimson Camera Technical Sales, Inc.

Dartmouth College
Delaware, University of
Dickinson College
Dillard University
Duke University
Duke University Medical Center

Eastern Pennsylvania Psychiatric Institute
Eisenhower College
Emmanuel College
Emory University
Emory University School of Medicine
Environmental Research Laboratory, Rhode Island
Evergreen State College, The
Eye Research Institute of the Retina Foundation, Boston

Florida, University of
Florida, University of, College of Medicine
Florida State University
Forest Products Laboratories
Fort Hays State University
Fred Hutchinson Cancer Research Center

General Electric Corporation
George Mason University
Georgia, University of

Gillette Company, The
Gray Seal Research Station
Gustavus Adolphus College

Hahnemann Hospital
Hahnemann Medical College
Hamamatsu Systems, Inc.
Hamilton College
Hampden-Sydney College
Hartwick College
Harvard Medical School
Harvard University
Hawaii, University of
Hawaii, University of, Kewalo Marine Laboratory
Hawaii, University of, Manoa
Hawaii, University of, Pacific Biomedical Research Center
Hofstra University
Holy Cross College
Houston, University of
Howard University
Howard University, College of Medicine
Hunter College

IBM
Ichthyological Associates, Inc.
Illinois State University
Illinois, University of
Illinois, University of, College of Medicine
Illinois Institute of Technology
Indiana University
Institute for Basic Research in Mental Retardation
Institute for Cancer Research, The
Iowa, University of
Iowa State University

JEOL-USA Inc.
John B. Pierce Foundation Laboratory
Johns Hopkins Hospital, Wiloner Clinic
Johns Hopkins University, The
Johns Hopkins University, The, School of Hygiene and Public Health
Johns Hopkins University, The, School of Medicine

Kansas, University of
Kentucky State University
Kentucky, University of
Kenyon College
Kevex Corporation
Knoxville College
Kresge Eye Institute

Laboratory for Comparative Biochemistry
Lafayette College
Leitz, E., Inc.
Le Moyne College
Louisiana State University
Louisville, University of

Macalester College
Maine, University of
Maryland, University of
Maryland, University of, School of Medicine
Massachusetts, University of, Amherst
Massachusetts, University of, Boston
Massachusetts, University of, Worcester
Massachusetts Eye and Ear Infirmary
Massachusetts General Hospital
Massachusetts Institute of Technology
Massachusetts State Lobster Hatchery
Mayo Clinic
McGill University
McMaster University
Medical College of Georgia
Medical College of Ohio
Merck and Company
Mercy College
Mercy Hospital
Miami, University of
Miami, University of, School of Medicine
Michigan, University of
Michigan State University, College of Veterinary Medicine
Mid-Maine Medical Center
Minnesota, University of
Miriam Hospital, Rhode Island
Monmouth Medical Center
Montefiore Hospital and Medical Center
Morehouse College
Morgan State University
Mount Holyoke College
Mount Sinai Medical Center, Milwaukee
Mount Sinai School of Medicine, New York
Murray State University

Nasson College
National Institute of Alcohol Abuse and Alcoholism
National Red Cross
National Institutes of Health
National Marine Fisheries Service/NOAA
National Park Services
Naval Submarine Medical Research Laboratory
Nebraska, University of, Omaha
New England Aquarium
New Hampshire, University of
New York, City University of, Brooklyn College

New York, City University of, City College
New York, City University of, Herbert Lehman College
New York, City University of, Hunter College
New York Medical College
New York, State University of, Albany
New York, State University of, Binghamton
New York, State University of, Buffalo
New York, State University of, Downstate Medical Center
New York, State University of, New Paltz
New York, State University of, Potsdam
New York, State University of, Purchase
New York, State University of, Stony Brook
New York, State University of, Syracuse
New York University School of Medicine
Nikon, Inc.
North Carolina, University of, Chapel Hill
North Carolina, University of, School of Medicine
North Carolina State University
North Dakota, University of, School of Medicine
Northeastern Illinois University
Northeastern University
Northeast Ohio University College of Medicine
Northwestern University
Northwestern University, Dental School
Northwestern University, Medical School
Notre Dame, University of
NOVA Biomedical
Numonics Corporation

Oakland University
Oberlin College
Ohio State University
Ohio University
Ohio University College of Medicine
Oklahoma State University
Oklahoma, University of
Olympus Corporation of America
Opti-Quip, Inc.
Oregon, University of
Oregon State University

Pennsylvania, University of
Pennsylvania, University of, School of Medicine
Pennsylvania Medical College
Pennsylvania State University
Philips Exeter Academy
Pittsburgh, University of
Plattsburgh State University College
Plum Island Animal Disease Center
Polaroid Corporation
Pomona College
Princeton University
Public Health Research Institute
Purdue University

Quinnipiac College

Rainbow Babies and Children's Hospital
Rainin Instrument Company, Inc.
Rhode Island College
Rhode Island Hospital
Rhode Island, University of
Rice University
Rochester, University of
Rochester, University of, School of Medicine
Rockefeller Foundation
Rockefeller University, The
Roosevelt University
Rush Medical College
Rush-Presbyterian-St. Luke's Medical Center
Rutgers—The State University of New Jersey
Rutgers University Medical School

St. Peter's College
St. Rose, College of
St. Vincent's Hospital
San Jose State University
Sarah Lawrence College
Savannah River Ecology Program
Scripps Institute of Oceanography
Seiler Instrument and Manufacturing Company, Inc.
Sidney Farber Cancer Institute
Sloan-Kettering Institute
Spelman College
Smithsonian Institution
South Carolina, University of
South Florida, University of
Southern Mississippi, University of
Stanford University
Stanford University Medical Center
Stanford University, Hopkins Marine Center
Stetson University
Swarthmore College
Syracuse University
Systemculture Corporation

Tams Engineers & Architect
Temple University
Temple University Medical School
Texas, University of, Arlington
Texas, University of, Austin
Texas, University of, Galveston

Texas, University of, Health Center, Dallas
Texas Christian University
Texas Southern University
Texas Technical University
Tougaloo College
Tousimis Research Corporation
Trinity College
Tufts University
Tulane University

Uniformed Services University of the Health Sciences
Union College
United States Department of Agriculture
United States Environmental Protection Agency
Utah, University of

Vanderbilt University, School of Medicine
Vassar College
Venus Scientific, Inc.
Vermont, University of
Veteran's Administration Medical Center of Albany
Veteran's Administration Medical Center of Boston
Villanova, University of
Virginia, University of
Virginia, University of School of Medicine
Virginia Polytechnic Institute
Virginia State University

Washington, University of
Washington University
Washington University, School of Medicine
Wayne State University
Wayne State University, School of Medicine
Wellesley College
Wells College
Wesleyan University
Westat Research Corporation
West Chester State College
Wheaton College
William Paterson College
Williams College
Wisconsin, University of
Wisconsin, University of, Madison
Wistar Institute
Woods Hole Engineering Association
Woods Hole Oceanographic Institution
Worcester Foundation for Experimental Biology
World Wildlife Fund

Yale University
Yale University, School of Medicine

FOREIGN

Aarhus, University of, Denmark
Alberta, University of, Canada
Athens, University of, Greece
Basel, The University of, Switzerland
Bedford, Institute of Oceanography, Canada
Berlin, Free University of, West Germany
Biomedical Research Center for Infectious Diseases, Egypt
British Cŏlumbia, University of, Canada
Calgary, University of, Canada
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XIII. LABORATORY SUPPORT STAFF

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Smith, Homer P., General Manager

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Walsh, Michael P.

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Valanti, Iris

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Haskell, Barbara, Public Relations Officer
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Mountford, Rebecca
Schwartz, Susan

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Broderick, Madeline
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Smart, Merilyn A.
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Ward, Frederick
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Clark, Diana
Clark, Sarah
Coombs, Roxane
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Frank, Charlotte F.
Grice, Joan H.
Hough, Nancy L.
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Laderman, Rachel
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Trapasso, Bruno
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Barnes, John S.
Eberhard, Carol A.
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Golder, Robert J.
Kerr, Louis
Makredes, Nicholas
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Sylvia, Frank E.

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Bartholomew, Polly

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Brinkman, Paula
Burnett, Lynn
Campbell, Lisa
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Lauther, Gary
Lee, James
Levenbaum, Ronald
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Rooney, Colleen
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Sullivan, Gail
Travis, Mark
Valois, Francis
Walker, Allen
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Wuethrich, Lisa

Reference: *Biol. Bull.* **161**: 98–103. (August, 1981)

A LYTIC MOLECULE ACTIVE AGAINST A CILIATE DURING A TRANSMISSIBLE DISEASE OF *SIPUNCULUS NUDUS*[1]

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Station Biologique, Roscoff, France, and The Johns Hopkins University, Baltimore, Maryland[2]

ABSTRACT

A pox-like disease of *Sipunculus nudus* was transmitted experimentally and serially by scarifying the skin of normal specimens of *Sipunculus* and exposing them in an open dish of running seawater to spontaneously infected animals. Animals allowed to repenetrate the sand during the incubation period did not develop signs of infection.

A lysin which destroys ciliates (*Anophrys*), obtained from crab blood, appeared about 4–6 days after infection and persisted until death of the animal at 16–20 days from ulceration and secondary infection. The lysin was stabilized by mixing the cell-free fluid with 0.01 *M* EDTA, and full activity was recoverable in one peak on Sephadex-200 gel. The molecular weight was estimated as about 250,000 daltons.

INTRODUCTION

Invertebrates have a variety of immune reactions (Shope and Maramorosch, 1975) and a greater variety of parasites (Kinne, 1980). Although invertebrates lack "antibody," they do have several serum substances that appear in response to disease. Among these substances is a lysin which appears in the blood of *Sipunculus nudus* (a marine coelomate) following the injection of a ciliate or in the course of a pox disease of the skin. This substance rapidly lyses the marine ciliate, *Anophrys*, thus providing a convenient *in vitro* test for activity. Since the reaction leads to complete lysis of the ciliate, it should eventually be examined in the context of evolution of the complement cascade in vertebrates.

Bang (1966) described the appearance and disappearance within the serum of *S. nudus* of a high titer of a lytic substance following the injection of large amounts of either *Anophrys* or certain gram-negative marine bacteria. At the same time, some animals used for other purposes in colonies of *S. nudus* maintained in aquaria at the Station Biologique developed a spontaneous skin disease characterized by pocks and ulcerations. This was followed by the development of the lysin. In subsequent summers at Roscoff, the disease was again noticed in the laboratory colonies, especially if the animals were kept in running seawater without the 6-in substrate of sand in which they normally burrow. High titers of lysin were present on each of three occasions when the disease occurred. This report presents data on: (i) production of the experimental disease following scarification; (ii) the continued association of the lysin with the disease; and (iii) the stabilization and beginning characterization of the lytic molecule.

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Abbreviations used: R cells—presumptive regulatory cells; EDTA—ethylenediaminetetraacetate.

[1] Supported in part by NIH grant #5P 50HL-19157.

[2] Address for reprints.

MATERIALS AND METHODS

To test for lysin, *Anophrys*-infected *Cancer pagurus* or *Carcinus maenas* crabs were maintained in the laboratory. Ten μl of crab blood containing approximately 200 *Anophrys* specimens were placed beside 5 μl of test serum on a microscope slide. The drops were mixed rapidly with a needle. Five min after mixing, morphology and mobility of *Anophrys* were examined microscopically. Rounded organisms with indistinct edges were judged to be lysed. All tests were performed at room temperature.

The titer of the lysin was roughly quantitated as in Bang (1966) on the basis of rapidity and degree of lysis within 15 min. In the fractionation studies of the lysin (see below), the individual pools of 4 drops were saved and the positive ones retested within 2 h. The amounts of lysin varied about 10–20% from the first to the second test. This did not affect the titer as recorded.

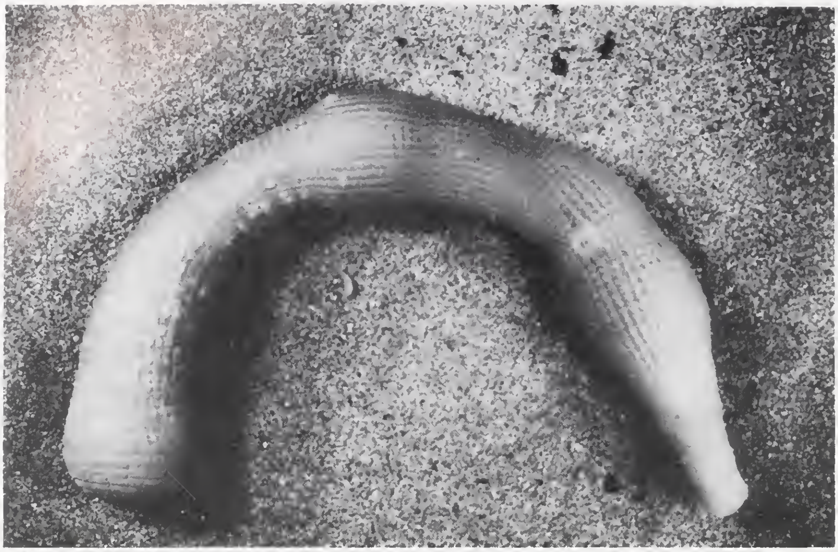
To obtain a constant supply of high titer lysin for the characterization studies (see below), the skin of normal *Sipunculus* specimens was scarified, and the scarified animals were placed in direct contact with a spontaneously infected animal in running water without sand. For this purpose, a total of 35 animals, all of which had been kept in the aquarium away from the experimental animals, and which showed no spontaneous disease, were used. All had had access to sand and had remained buried under the sand. They were scarified with a sharp new needle by making light cross-hatched marks on their posterior ends, with the marks extending down into the corrugated skin of their sides. After this, the animals were placed in glass jars or plastic boxes with running water but minimal sand, and in direct exposure to infected animals. At the start, the infected animal was a spontaneously infected one. By the end of the summer, about five serial passages of the infection had taken place.

In the original study (Bang, 1966), the lysin was found to be highly heat-labile, and was destroyed by ether. When frozen at −20°C, the lysin maintained some activity for 2–3 weeks, but at room temperature or at 4°C it was quite labile, so that characterization was difficult. In the present experiments, the addition of 0.01 *M* EDTA (ethylenediaminetetraacetate) considerably stabilized the activity and enabled us to carry out gel filtration studies. A column (0.5 cm inside diameter × 7 cm height) was packed with Sephadex G-200 beads and equilibrated in boiled filtered seawater containing 0.01 *M* EDTA at room temperature. One hundred μl of lysin was applied to the column and fractionated at a flow rate of 5 ml/h. The individual drops are recorded as fraction numbers. Four drops were collected to obtain a sufficient volume and assayed for lytic activity. Markers used were blue dextran (Pharmacia) and catalase (Sigma, molecular weight 232,000 daltons).

RESULTS

Figure 1 shows the typical pock-like lesion of the early spontaneous disease. This, with time, progresses to ulceration. Small biflagellate protozoa (possible dinoflagellates) are often found in the lesions (Bang, 1966), but it is not clear whether they are primary or secondary invaders. Thus the etiological agent is undetermined.

Pock-like lesions were routinely produced along the lines of scarification in 13/14 animals that were scarified and left free of sand cover (Table I). The lesions appeared about 6–8 days after scarification. The lysin also was first detected in the blood at about the same time, but in several instances the lysin appeared a day or so before the lesions or vice-versa. Thus, a direct correlation between the two events was not established.



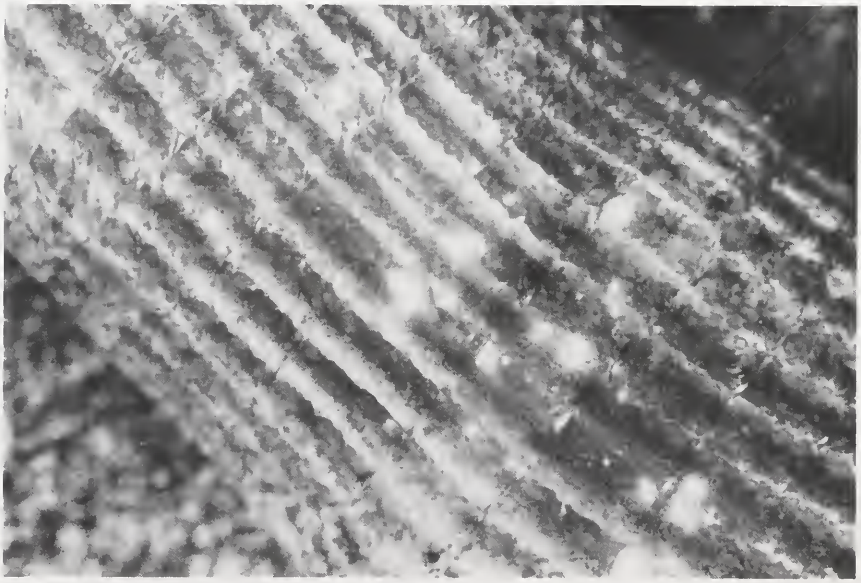


FIGURE 1a (above). Scattered spontaneous pox lesions on posterior region of *Sipunculus nudus*; ½ × natural size. 1b (below) Spontaneous lesions of same animal; 3 × natural size. The lesions occur both between the muscular ridges and on top of them.

Neither lesions nor persistent lysin appeared in animals scarified, exposed, and allowed to go under the sand. In one final experiment (Table I, #12), seven animals were scarified, exposed to infected animals for 1, 2, or 3 days, and then allowed to burrow under the sand. In these, no significant high-titer lysin appeared, even though initial small lesions appeared as early as 3 days in the animals kept free of sand for 3 days. Lesions disappeared thereafter. The positive control animal, which was scarified and kept in open exposure, developed lesions and lysin. In another experiment, one animal scarified and kept out of sand for 17 days developed

Table I

Summary of experimental transmission of pox disease of Sipunculus. *Injection was by intracoelomic injection of scrapings. d = days.*

Expt.	No. of animals inoculated	Mode of maintenance	Mode of inoculation	Results
# 2	2	no sand	scarification	pox 6 d, lysin 8 d, 1 dead 22 d, 1 killed 18 d
# 3	1	no sand	scarification	lysin 4 d, no pox, dead 6 d
# 4	2	no sand	scarification	small lesions 6 d, lysin 12 d
# 5	2	sand	injection	no lesions, irregular lysin 2–9 d, recovery 10 d
# 6	2	no sand	injection	no lesion, poor lysin response
# 7	2	no sand	scarification	pox and lysin 6 d, killed 16 d
# 8	2	no sand	scarification	lysin 5–8 d, no lesinos, recovery
	2	no sand	scarification	lysin and pox 5 d in one, other one not infected
# 9	2	no sand	scarification	pox and lysin 6 d
#10	4	sand	injection	no lesion, irregular lysin
#11	2	no sand	scarification	pox 7 d, lysin 9 d
#12	7	put in sand at intervals (see text), 1 kept out of sand	scarification	see text

sharply demarcated lesions and lysin by day 6. The animal then was allowed to go under the sand on the 17th day (Fig. 2, #27). Within 3 to 4 days, the external lesions disappeared, but the lysin persisted at high titer for 11 days after reestablishment of life under the sand, at which time the study was discontinued.

Injection of a suspension of scrapings from the skin lesion (Table I, experiments #5, #6, #10; total of 8 animals) caused temporary change in the lysin (possibly due to the contaminating bacteria), but no external lesions were apparent regardless of whether the animals were buried under the sand or exposed to running seawater.

The size of the specialized R cells on the circulating urn cell complex changed in several of the animals which developed skin lesions and lysin. These changes consisted of hypertrophy, elongation, and the "spontaneous" secretion of clear mucus apparently directly from these cells, and unassociated with the usual long tails of secretion seen when the urn cell complex is stimulated *in vitro* to secrete mucus tails. The regular production of a manifest pox disease following experimental exposure made it possible to follow the course of the serological reaction concomitantly with the overt disease. As Figure 2 shows, the lysin appeared 4–8 days after scarification and exposure, and was maintained thereafter in exposed animals.

As shown in Figure 3, lysin activity fractionated on a Sephadex G-200 column emerged as a single peak. Its molecular weight, as approximated by Andrews' method (Andrews, 1965) was 250,000 daltons. It is, however, possible that a more detailed analysis of this peak might show more than one component.

Discussion

Although the infection occurs spontaneously in occasional animals left free of sand, and can now routinely be transferred from one animal to another, its etiology is not settled. A small protozoan, which is unidentified, appearing somewhat like

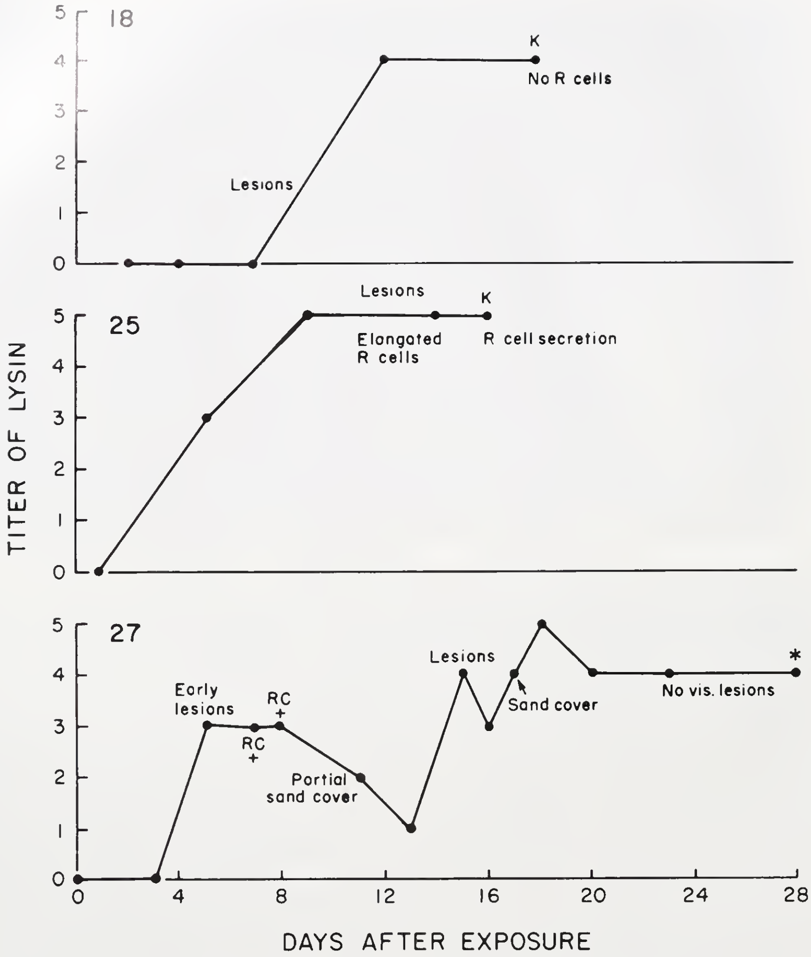


FIGURE 2. Course of disease symptoms and cellular reaction in typical pox disease induced by scarification and exposure. Each chart represents an individually numbered animal which had been scarified and exposed to a previously infected animal. The time of development of lesions was roughly correlated with the appearance of the lysin, but both varied by a few days. Animal #27 illustrates the decrease in lesions and lysin during early period under the sand, the reappearance of both under partial cover, and disappearance of lesions but persistence of lysin with complete cover. K = killed; RC = R cells positive for secretion; * = cured, but experiment discontinued.

a euglenoid, was found frequently but not invariably in scrapings of the lesion, even when searched for repeatedly in early typical lesions. Thus the term "pox disease" refers only to the type of lesions, and the question of a virus etiology is untested.

The regular appearance of lytic activity in the course of this infection, the finding that the lysin can be stabilized in the presence of 0.01 *M* EDTA, and the likelihood that the activity is contained in one molecule, now raises the question of relationship of the lysin to the complement cascade in vertebrates. This cascade has two pathways. The alternate one unassociated with the antibody is presumably the more ancient phylogenetically. In the *S. nudus* lysin, the large molecule acts directly on *Anophrys* obtained from infected crab blood. When this lysin was tested on cultured ciliates of other species (unpublished), results were not as striking. The

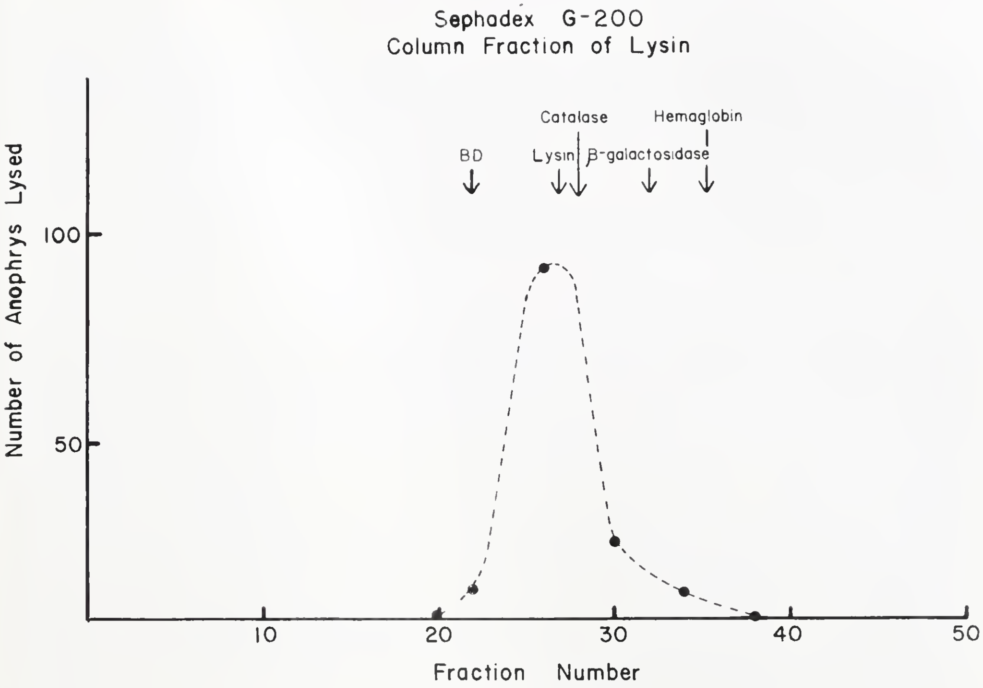


FIGURE 3. Fractionation of lysin from serum of spontaneously infected animals.

ciliates in crab blood may be "prepared" in some way for the *S. nudus* lysin, even though *Cancer* and *Carcinus* are unable to lyse them, since other crab species do at times produce a serum factor which clumps *Anophrys* and in rare cases lyses them (Bang, 1967).

The cellular source of the lytic molecule is unknown. It was originally shown that rapid but temporary release of the lysin occurred when *Sipunculus* blood was put in contact *in vitro* with foreign substances including crab blood containing *Anophrys* (Bang, 1966). This early lysin, however, may be separate from the persistent lysin here described. Possible sources are the clusters of secretory R cells within the autologous urn cell complexes (Bang and Bang, 1980), which swim freely in the *S. nudus* coelom. When high titers of lysin are present, the R cells hypertrophy and produce increased amounts of secretion (Bang and Bang, unpublished). The role of host amebocytes, or of the enigmatic plateletlike vesicles which enclose a fluid matrix, cannot be excluded.

LITERATURE CITED

ANDREWS, P. 1965. The gel-filtration behaviour of proteins related to their molecular weights over a wide range. *Biochem. J.* **96:** 595–606.

BANG, B. G., AND F. B. BANG. 1980. The urn cell complex of *Sipunculus nudus*: a model for study of mucus-stimulating substances. *Biol. Bull.* **159:** 571–581.

BANG, F. B. 1966. Serologic response in a marine worm, *Sipunculus nudus. J. Immunol.* **96:** 960–972.

BANG, F. B. 1967. Serologic responses among invertebrates other than insects. *Fed. Proc.* **26:** 1680–1684.

KINNE, O. 1980. *Diseases of marine animals. I. General aspects, Protozoa to Gastropoda.* John Wiley, Somerset, N. J. 460 pp.

SHOPE, R. E., AND K. MARAMOROSCH, EDS. 1975. *Invertebrate immunity.* Academic Press, New York. 365 pp.

Reference: *Biol. Bull.* **161**: 104–114. (August, 1981)

CELLULAR DEFENSE REACTIONS OF *PHASCOLOSOMA AGASSIZII*, A SIPUNCULAN WORM: PHAGOCYTOSIS BY GRANULOCYTES

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ABSTRACT

Based on Wright's blood stain, uninucleate circulating granulocytes of the sipunculan *Phascolosoma agassizii* Keferstein are of two types: acidophils and basophils. The granulocytes are characterized by numerous granules scattered throughout the cytoplasm. Morphology of the granulocytes viewed with the light and electron microscope is illustrated and described. The granules of the granulocytes contain the enzymes acid phosphatase, alkaline phosphatase, lipase, and peroxidase. Fibrin-like strands entrap foreign particles—polystyrene latex beads and *Staphylococcus aureus*—introduced into the coelomic fluid *in vivo* or *in vitro.* Granulocytes accumulate in the vicinity of the trapped foreign particles. Both acidophilic and basophilic granulocytes phagocytize polystyrene latex beads and *Staphylococcus aureus in vivo* and *in vitro.* The cytoplasmic granules of the granulocytes degranulate into the phagocytic vacuoles and new granules form from the concave face of the Golgi complex.

INTRODUCTION

The phylum Sipuncula contains unsegmented marine worm-like animals that have a muscular body enclosing a coelomic cavity. The cavity contains the internal organs and a variety of circulating cell types: (1) hemocytes, nucleated cells containing the respiratory pigment hemerythrin, (2) uninucleate granulocytes, also called amoebocytes or leukocytes, (3) cell pairs—"cell within a cell" structures also containing granules (Dybas, 1975), (4) enigmatic vesicles—very large multinucleate or multicellular masses often surrounding a vesicle (Hyman, 1959), (5) ciliated urns, (6) immature cells that appear to be differentiating into other cell types, and (7) ova or spermatids (depending on the animal's sex) shed from the gonads into the coelomic cavity, where they develop. Some species lack one or more of these cell types.

Sipunculans are hardy animals capable of withstanding laboratory conditions and repeated injections and bleedings. Therefore, they have been used as experimental animals to investigate cellular and humoral immune reactions. In *in vivo* and *in vitro* studies, the granulocytes have been observed to be the main phagocytic cells in *Sipunculus nudus* (Cuénot, 1900; Cantacuzène, 1922; Volkonsky, 1933; Valembois and Boiledieu, 1980), *Phascolosoma vulgare* (Volkonsky, 1933), and *Phascolosoma agassizii* (Towle, 1962; Blitz, 1965). However, Brown and Winterbottom (1969) injected thorium dioxide into the coelomic cavity of *Golfingia capensis*, a species without ciliated urns, and observed that all coelomic cells ingested the thorium particles. Triplett *et al.* (1958) found that both autograph and homograph tentacle transplants into the coelomic cavity of *Dendrostomum zostericolum* became encapsulated. However, neither phagocytosis nor encapsulation was

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enhanced when second transplants were made. They concluded, therefore, that *D. zostericolum* lacked the capacity to synthesize antibody to a specific antigen. In another study (Cushing and Boraker, 1975), coelomocytes from *D. zostericolum* were capable of distinguishing "self" from "not self." Homologous eggs treated by heating, staining, or sonification were recognized as "not self" and encapsulated. Male sipunculan coelomocytes did not encapsulate untreated homologous eggs injected into their coelomic cavity; however, eggs possessed some antigens which also occurred on sperm.

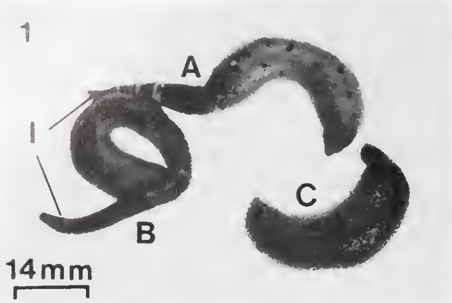
Other coelomocyte types have also been associated with specific immunological reactions. In *S. nudus*, the ciliated urns secrete mucus that agglutinates and traps the foreign particles (Cantacuzène, 1922; Bang and Bang, 1962, 1965, 1974, 1975, 1976). Agranular or hyaline leukocytes of *S. nudus* have a cytotoxic effect against xenogenic hemocytes. Lysis requires close contact between the cells (Boiledieu and Valembois, 1977; Valembois and Boiledieu, 1980). Hemocytes of *D. zostericolum* have antigens capable of reacting with various human antisera (Cushing *et al.*, 1963).

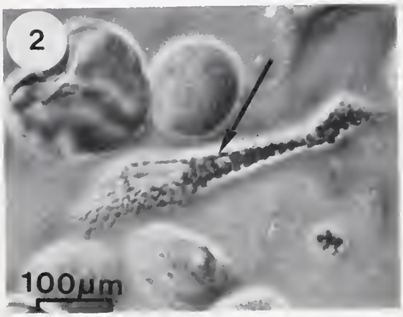
Strong anti-bacterial properties have been demonstrated in the coelomic fluid of *S. nudus* (Bang and Bang, 1962), *Phascolosoma gouldii* (Bang and Krassner, 1958; Krassner, 1963), *Golfingia gouldii* (Rabin and Bang, 1964; Krassner and Florey, 1970), *P. agassizii* (Blitz, 1965), *D. zostericolum* (Johnson and Chapman, 1970), and *Dendrostomum pyroides* (Krassner and Florey, 1970). Injecting bacteria in *D. zostericolum* resulted in production of a non-specific bactericidin not present, or present in very low amounts, in non-injected animals (Evans *et al.*, 1969; Evans *et al.*, 1973). Injecting ciliates or bacteria caused rapid production of a lysin for some bacteria, ciliates, and blood cells in *S. nudus* (Cantacuzène, 1922; F. B. Bang, 1966, 1967), and *P. agassizii* (Blitz, 1965). Although *P. agassizii* contained a lysin for some gram positive and gram negative bacteria, it does not contain the lysin for the ciliate protozoan that Bang and Bang (1962) observed in *S. nudus* (Blitz, 1965). Cushing *et al.* (1969) showed that a substance they called stop factor (SF) in the coelomic fluid of *D. zostericolum* could immobilize a marine dinoflagellate within 10 min; recovery took 12 h. Weinheimer *et al.* (1970), demonstrated a naturally occurring lysin and hemagglutin for foreign red blood cells in the coelomic fluid of *D. zostericolum*. Foreign particles were also agglutinated in the coelomic fluid of *S. nudus* (Cantacuzène, 1922) and *P. agassizii* (Towle, 1962; Blitz, 1965). The bactericidins, lysins, and agglutinins can be separated from one another on the basis of characteristic properties, such as thermostability, enzyme sensitivity, and filtration.

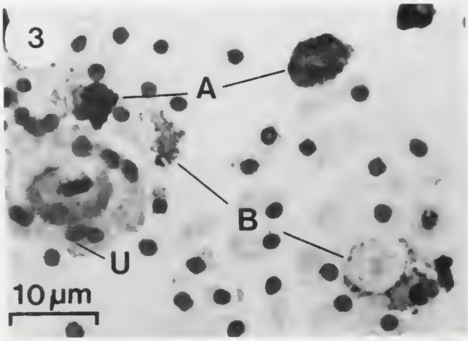
The studies cited above show that sipunculans can launch both cellular (phagocytosis and encapsulation) and humoral (agglutinins, lysin, and bactericidin production) defense reactions. With the exception of the ciliated urn, which has been studied in some detail in *Phascolosoma scolops* (Ohuye *et al.*, 1961), *S. nudus* (Bang and Bang, 1962, 1965, 1974, 1975, 1976; Nicosia, 1979), and *P. agassizii* (Dybas, 1976), little is known about the specific functional roles or detailed morphology of the defense system. This study was undertaken to elucidate the role of the circulating granulocytes in the cellular immune response of *Phascolosoma agassizii.*

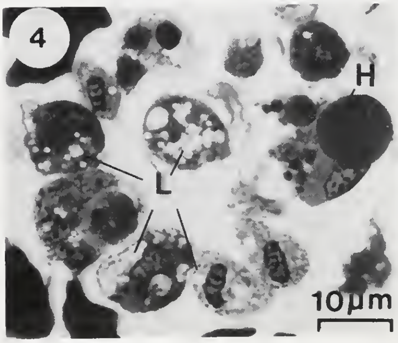
MATERIALS AND METHODS

The 30 specimens of *Phascolosoma agassizii* Keferstein (Fig. 1) used in this study were collected from crevices in rocks exposed at low tide along Arroyo de









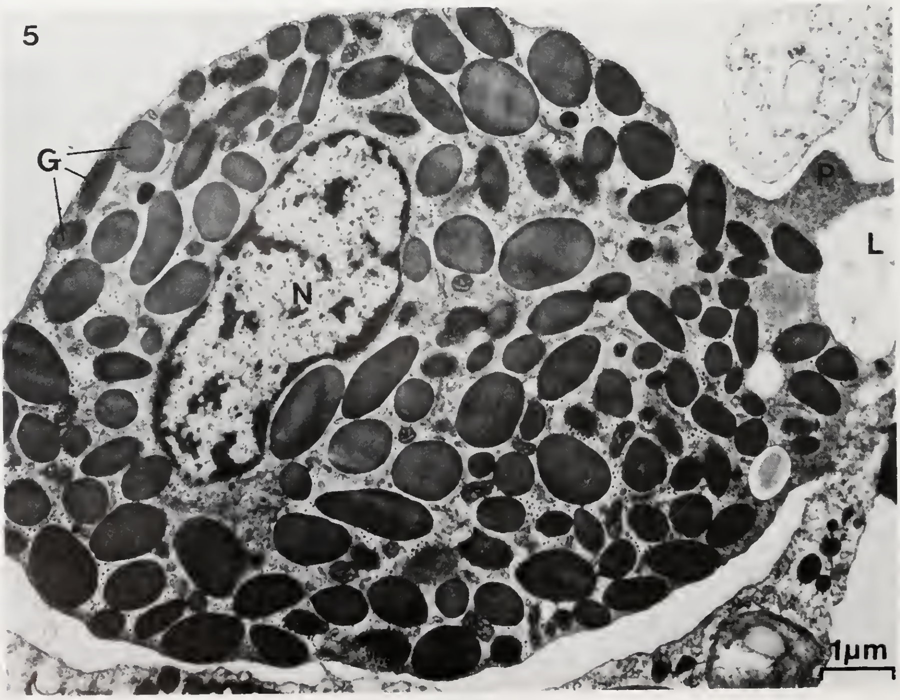


FIGURE 1. Whole specimens of *Phascolosoma agassizii*, A and B with introverts (I) extended, C with introvert retracted, exhibiting the characteristic "peanut" shape.

Frijoles State Beach, California. By placing the sipunculans on a dental wax sheet and cutting off the posterior 2–3 mm with an alcohol-cleaned razor blade, a large drop of coelomic fluid was obtained. This was pipetted into various solutions or quickly immersed in fixative.

Whole-cell preparations for light microscopy were made using a Shandon cytocentrifuge (Shandon Instr. Co., Sewickley, Penn.) designed for centrifuging cell suspensions directly on the slide. Cell suspensions were made from pooled samples of coelomic fluid (10 animals/sample). All tests were performed in triplicate. The slides were either air-dried and stained with Wright's blood stain (Lillie, 1969) or reacted for one of four different enzymes: (1) Goodpasture's reaction for peroxidase, omitting the fixative (1919), (2) Barka and Anderson's (1962) reaction for acid phosphatase, extending the incubation times from 45 min to 2–4 h, (3) Burstone's (1958) reaction for alkaline phosphatase, extending the incubation time to 4 h, or (4) Gomori's reaction for lipase from Yam *et al.* (1971). Material known to give a positive reaction for each enzyme (human bone marrow or rat kidney) was tested simultaneously with each sipunculan test sample. In other controls, the substrate or capture agent was omitted, or an inhibitor was added to the incubation medium: 0.01 *M* NaF for acid phosphatase, 0.001 *M* cysteine for alkaline phosphatase, and 0.01 *M* KCN for peroxidase (Bainton and Farquhar, 1968). After the enzyme reactions were completed, the slides were counterstained to enhance coelomocyte recognition (except for the acid phosphatase preparations, in which the reaction product was scant and faint), coverslipped, and examined under the light microscope. For a reaction to be classified as positive for an enzyme, the positive controls also had to be positive and the negative controls—both omission of substrate and addition of inhibitor—had to be negative.

For electron microscopy and light microscopy of Araldite-embedded coelomocytes, cells were fixed in 3% distilled glutaraldehyde buffered with 0.2 *M* sodium cacodylate in millipore-filtered seawater. After overnight fixation, cells were postfixed in 1% Michaelis-buffered osmium tetroxide (Caufield, 1957) for 90 min, dehydrated in graded ethanols, and embedded in Araldite (Luft, 1961). To facilitate handling, cells were packed by centrifuging (about 10,000 × *g*) with a Microfuge 152 (Beckman Instr., Inc., Spinco Div., Palo Alto, Calif.) (Malamed, 1963). Polymerized blocks were sectioned on a Sorvall Porter-Blum MT-2 Ultramicrotome (Dupont Corp., Norwalk, Conn.) equipped with glass knives.

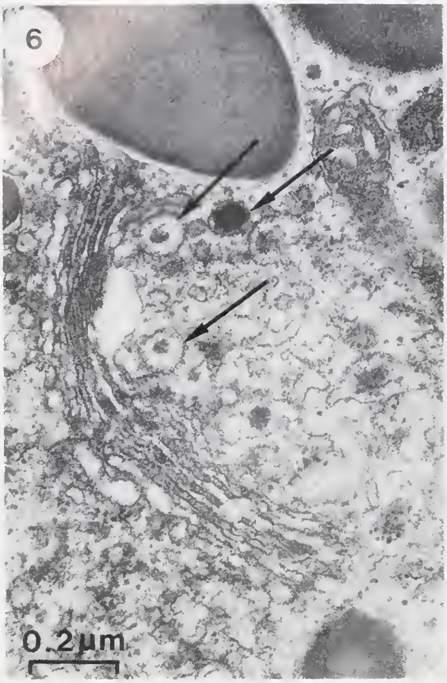
For light microscopy, 0.5 μm sections were stained with 0.5% toluidine blue in sodium borate (Trump *et al.*, 1951). For electron microscopy, sections were placed on Parlodion and carbon coated grids (Hayat, 1970), double stained with 1% alcoholic uranyl acetate (Watson, 1958) followed by lead citrate (Reynolds, 1963), and viewed with a Siemans Elmiskop IA. Electron micrographs were taken at original magnifications of 3000 to 30,000 times on Kodalith 70 mm Estar base film (Eastman Kodak Co., Rochester, N. Y.).

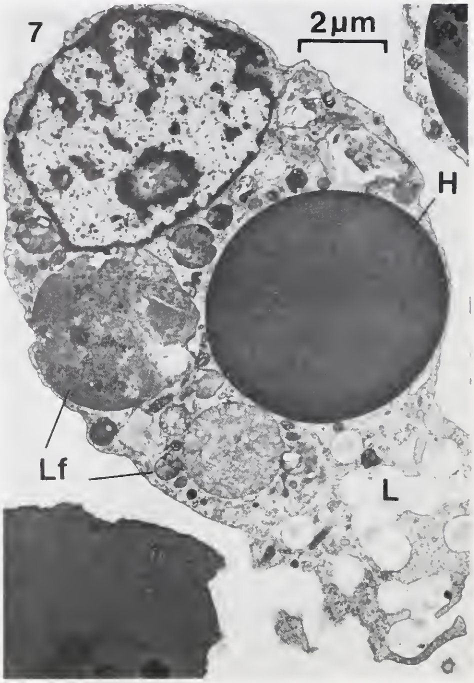
FIGURE 2. Phase contrast micrograph of an unfixed amoeboid granulocyte (arrow) showing the nucleus and cytoplasmic granules.

FIGURE 3. Whole air-dried coelomocytes stained with Wright's blood stain: acidophilic granulocytes (A), basophilic granulocytes (B), and a ciliated urn (U). The other cells in the field of view are hemocytes.

FIGURE 4. Light micrograph of several granulocytes that have phagocytized latex beads (L) and one that has phagocytized a hemocyte (H). Thick Araldite section stained with toluidine blue.

FIGURE 5. Fine structure of a typical granulocyte: nucleus (N), granules (G), pseudopodia (P) partially surrounding latex beads (L). Glycogen, rough endoplasmic reticulum, and mitochondria are dispersed throughout the cytoplasm.





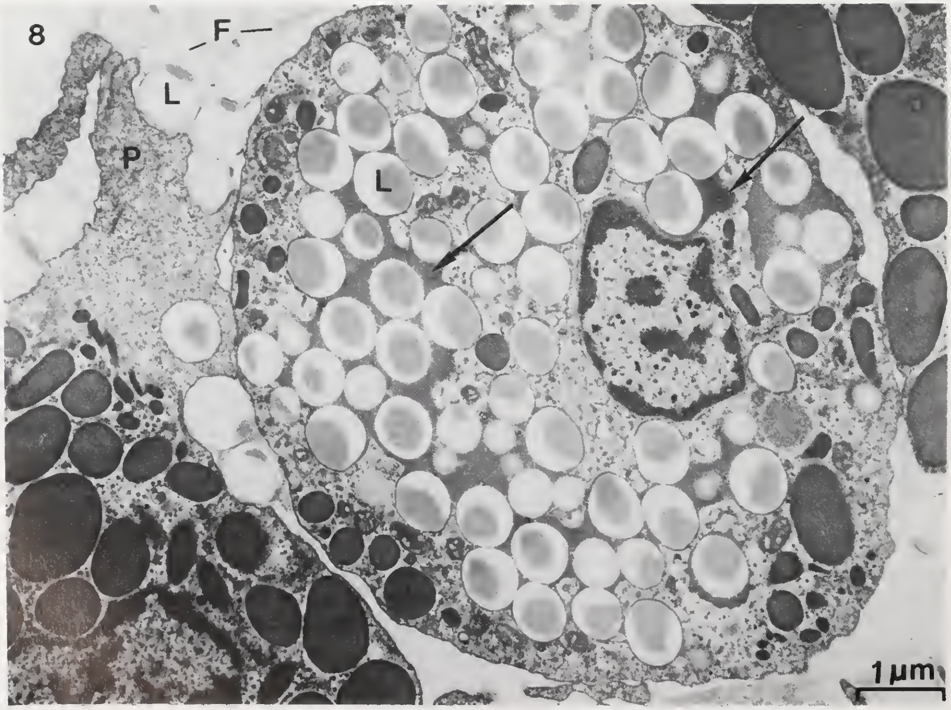


FIGURE 6. High magnification electron micrograph of a granulocyte 60 min after *in vitro* incubation with latex beads. New granules (arrows) are forming off the concave face of the Golgi complex.

For determining phagocytic cells, both *in vivo* and *in vitro* experiments were performed using polystyrene latex beads, size 0.79 μm, and heat-killed *Staphylococcus aureus* 502A, size 1.0 μm. In *in vivo* experiments, particles suspended in millipore-filtered seawater were injected in amounts of 0.1 cc through the body wall into the coelomic cavity. In *in vitro* experiments, the particles suspended in seawater were added to a test tube containing whole coelomic fluid at a concentration of approximately 10,000 bacteria or latex beads per milliliter of coelomic fluid. At 7, 30, and 60 min, samples of whole coelomic fluid were processed as previously described for electron microscopy. Other samples were viewed as living cells with the light microscope.

RESULTS

In unfixed living cells (Fig. 2), some granulocytes (10.95 μm in diameter) ranged from colorless to pale green, and round to amoeboid in shape. Others, slightly larger (15.33 μm in diameter), contained granules varying from colorless to dark gold or orange.

In Wright's blood stain (Fig. 3), the granulocytes had either basophilic or acidophilic granules, but no single cell showed both staining reactions simultaneously. The size and number of granules varied. The cytoplasm of some cells was completely filled with granules, whereas in others granules were dispersed through the cytoplasm. In general, the smaller granulocytes contained numerous acidophilic granules, while the larger granulocytes contained basophilic granules. The basophilic granules varied more in size and in number than did the acidophilic granules.

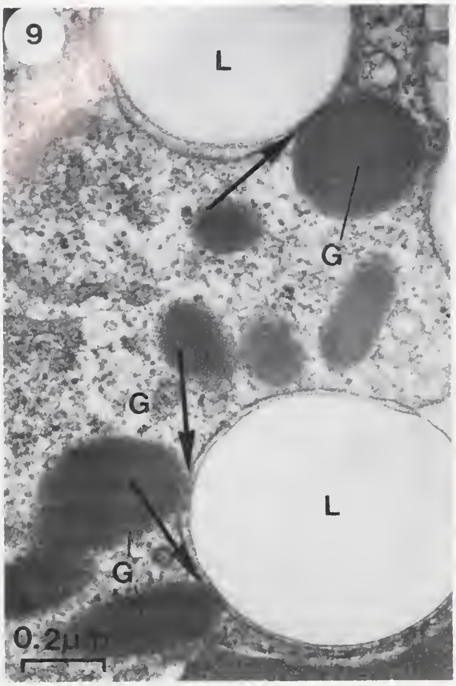
In toluidine-blue stained sections (Fig. 4), the granules in the granulocytes all stained a uniform blue, regardless of size. Pseudopodia of amoeboid granulocytes were free of granules. The cytoplasm often contained phagocytized latex beads or *S. aureus* and what appeared to be phagocytized hemocytes.

In enzyme studies reactions were positive for acid phosphatase, alkaline phosphatase, lipase, and peroxidase. The positive reaction product was localized in the granules of the granulocytes. Attempts to stain for more than one enzyme on a slide were not successful.

The electron-dense granules in the granulocytes were the most distinguishing feature of the cells revealed by electron microscopy (Figs. 5–7). The granules all displayed a uniform density except in the immediate vicinity of a Golgi complex, where they showed a clear zone between the limiting membrane of the granule and its electron-dense core (Fig. 6). A nucleolus was occasionally encountered. Ribosomes were found along the perinuclear membrane, and rough and smooth endoplasmic reticulum was dispersed throughout the cytoplasm. Mitochondria, glycogen, and lipid droplets were also present. In many cells, residual bodies, lipofuscin-like pigments, and membrane whorls could be seen in the cytoplasm. Cell organelles or other cells were occasionally recognizable within a phagocytic vacuole (Fig. 7).

FIGURE 7. Fine structure of a granulocyte with varied cytoplasmic inclusions: lipofuscin-like material (Lf) which is thought to be insoluble residues of phagocytosis, a phagocytized hemocyte (H), and latex beads (L).

FIGURE 8. Electron micrograph of granulocytes 60 min after *in vitro* incubation with latex beads. In this plane of section, 74 latex beads (L) are visible within one cell, many within a single phagocytic vacuole. Most granules have already degranulated into the phagosome. The contents of the degranulated granules appears as dark material in the phagosome (arrows). Extracellular latex beads are trapped in a fibrin-like clot (F). Granule-free pseudopodia (P) from the cell left of center are beginning to surround the extracellular latex beads.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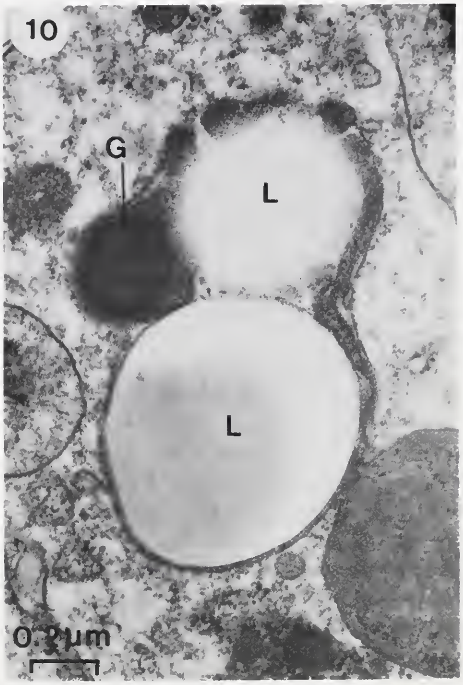

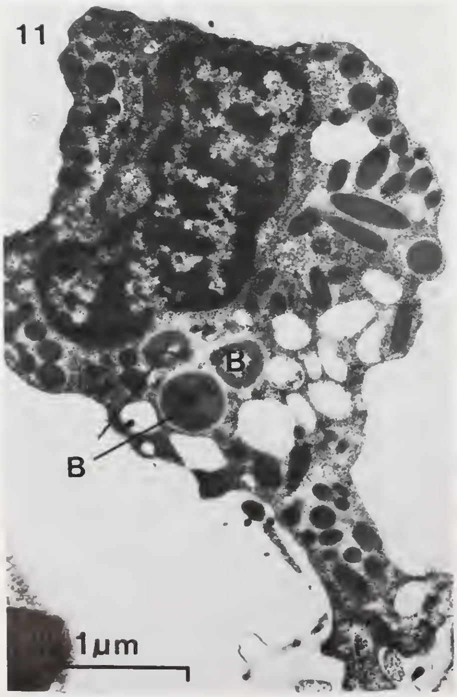

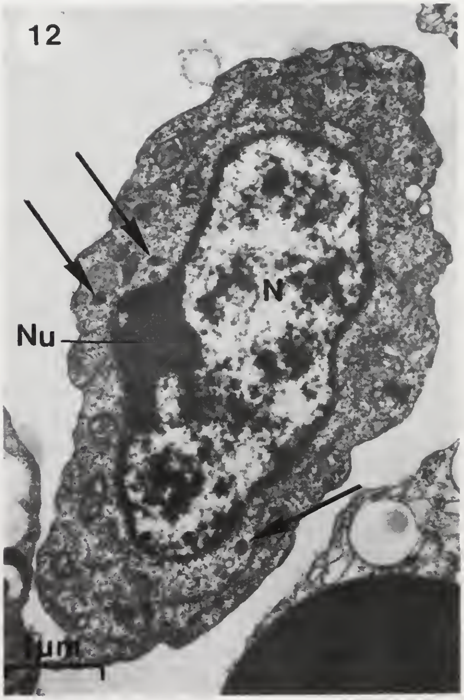

FIGURE 9. High magnification electron micrograph of a granulocyte 7 min after *in vitro* incubation with latex beads. The limiting membranes surrounding the granules (G) have fused with the membranes surrounding the phagocytic vacuoles (arrows). Within the phagosomes are latex beads (L).

In phagocytosis experiments (Figs. 4, 5, and 7–11), the reactions of the granulocytes in *in vivo* or *in vitro* experiments did not differ drastically. Immediately after injection of polystyrene latex beads or *S. aureus*, clots of fibrin-like strands appeared around the injected particles, trapping them and preventing their rapid dispersal. By 7 min, granulocytes were near the clotted foreign particles and pseudopodia were surrounding a latex bead (Figs. 4, 5, 7, and 8) or bacterium. Granulocytes were the dominant cell type surrounding the clotted foreign particles (Fig. 4). Most granulocytes already had foreign particles within their phagocytic vacuoles. By 30 min, extensive degranulation into the phagocytic vacuoles was apparent. Granules surrounded the phagosomes, and membranes surrounding the granules fused with membranes of the phagosome (Fig. 9). The granule contents that empty into the phagosome were visible at first as discrete condensed spheres adjacent to the inner side of the vacuole membrane (Fig. 10). Later, the contents dispersed throughout the phagocytic vacuole (Fig. 8). There were often numerous latex beads within a phagocytic vacuole.

By contrast, even after 60 min, although numerous *S. aureus* specimens had been phagocytized, it was rare to find even two of them in a phagocytic vacuole (Fig. 11). By 60 min, many granulocytes were almost completely degranulated. Golgi complexes were prominent and new granules appeared to be forming from the concave face of the Golgi complex (Fig. 6). Occasionally, granulocytes degranulated outside cells, into a clot of foreign particles, usually into clots that were too large to ingest. Although some granulocytes contained no phagocytized particles, all mature granulocyte types, regardless of size, number of granules, or other large inclusions, were observed with phagocytized particles. Blast-like cells (Fig. 12) that contained a few dense granules, suggesting that they were an immature form of granulocytes, were never observed containing recognizable phagocytized particles.

Discussion

Coelomic cell types vary somewhat within the phylum Sipuncula, but granulocytes have been reported in all species in which the coelomic fluid has been studied. In *P. agassizii* all uninucleate granulocytes, regardless of variation in number and size of granules, can phagocytize polystyrene latex beads or *S. aureus in vivo* and *in vitro*. In *Sipunculus nudus*, where the ciliated urn is a mucus-secreting cell thought to be the primary agglutinator in trapping foreign particles, granulocytes (amoebocytes) surround the mucus tails of the urns, acting as scavengers phagocytizing the entrapped particles (Bang and Bang, 1965; 1971). In *P. agassizii*, after 30 min incubation with the foreign particles, urns and granulocytes often surrounded clotted foreign particles, but granulocytes were the dominant cell type. This was also observed by Blitz (1965).

In the present study, granulocytes seemed attached to the clotted material. They remained surrounding the clot throughout the manipulations required for processing

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FIGURE 10. After degranulation, granule contents (G) appear inside the phagosome adjacent to the latex beads (L).

FIGURE 11. Fine structure of a granulocyte 30 min after in vitro incubation with heat-killed *Staphylococcus aureus*. Three bacteria (B) have been phagocytized.

FIGURE 12. Electron micrograph of a blast-like cell, possibly an immature form of a granulocyte. The nucleus (N) contains a prominent nucleolus (Nu). A few granules (arrows) are scattered throughout the cytoplasm.

cells for electron microscopy. Foreign particles were also trapped in the current created by the beating cilia of the urns and phagocytized by the cupola cells of the ciliated urn (Dybas, 1976).

It is not known whether the fibrin-like strands which trap foreign particles in *P. agassizii* originate from components of the coelomic fluid, or if the presence of one of the numerous cell types is required. Regardless of the fibrin's origin, clotting, which prevents dissemination of particles throughout the entire coelomic cavity, would have survival advantage if the foreign particles were pathogens.

The phagocytic cells reacted positively for enzymes that in vertebrates are involved in digestion (acid phosphatase, alkaline phosphatase, and lipase) or in killing microbes (peroxidation of bacterial cell walls) (Klebanoff, 1967; Weiss, 1977). These enzymes may serve the same function in sipunculan granulocytes. The considerable diversity of granule size within a granulocyte could represent either different populations of granules or different stages in packaging and development.

Since Golgi complexes were prominent in degranulated granulocytes, and only rarely seen in non-degranulated granulocytes, degranulation may trigger the formation of new granules.

With the electron microscope, granulocytes were often seen to contain residual bodies or large inclusions of undigested material. The fate of these inclusions is not known. However, Towle (1962) observed in a phagocytosis experiment that carmine dye was excreted via the nephridia. He concluded that eventually exocytosis rids a cell of indigestable material. Cushing (1963—personal communication cited in Blitz, 1965) and Blitz (1965) also observed that foreign material was excreted via the nephridia.

I have no data on the granulocytes' life spans. Even after repeated bleedings, no granulocytes or other cell types were seen in mitosis. Blast-like cells that did not seem to belong to any recognizable cell type had some characteristics suggesting that they may represent an ontogenetic stage of the granulocytes. These cells were small (6 μm in diameter). They had a large nucleolus adjacent to the nuclear membrane; a basophilic cytoplasm containing mostly free ribosomes, but also some rough endoplasmic reticulum; mitochondria; and a few widely scattered granules. They never contained recognizable phagocytozed particles.

The sipunculan granulocytes resemble vertebrate neutrophils in structure and function, although no phylogenetic relationship between sipunculan and vertebrate immune responses has been shown. Nevertheless, to survive, sipunculans must have developed an efficient mechanism to recognize foreign substances, rid the open coelomic cavity of possible pathogens, and recycle nutrients from cells that are no longer functional.

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LITERATURE CITED

BAINTON, D. F., AND M. G. FARQUHAR. 1968. Differences in enzyme content of azurophil and specific granules of polymorphonuclear leukocytes. II. Cytochemistry and electron microscopy of bone marrow cells. *J. Cell Biol.* **39**: 299–317.

Bang, B. G., and F. B. Bang. 1965. Mucus hypersecretion in a normally isolated non-innervated cell. *Cah. Biol. Mar.* **6:** 257–264.

Bang, B. G., and F. B. Bang. 1971. Hypersecretion of mucus induced in isolated non-innervated cells. *Cah. Biol. Mar.* **12:** 3–10.

Bang, B. G., and F. B. Bang. 1974. Invertebrate model for study of macromolecular regulators of mucus secretion. *Lancet* **2:** 1292–1294.

Bang, B. G., and F. B. Bang. 1975. Cell recognition by mucus secreted by urn cell of *Sipunculus nudus. Nature* **253:** 634–635.

Bang, B. G., and F. B. Bang. 1976. The mucous secretory apparatus of the free urn cell of *Sipunculus nudus. Cah. Biol. Mar.* **17:** 423–432.

Bang, F. B., and B. G. Bang. 1962. Studies on sipunculan blood: Immunologic properties of coelomic fluid and morphology of "urn cells." *Cah. Biol. Mar* **3:** 363–374.

Bang, F. B. 1966. Serologic response in a marine worm, *Sipunculus nudus. J. Immunol.* **96:** 960–972.

Bang, F. B. 1967. Serological responses among invertebrates other than insects. *Fed. Proc.* **26:** 1680–1684.

Bang, F. B., and S. B. Krassner. 1958. Antibacterial activity of *Phascolosoma gouldii* blood. *Biol. Bull.* **115:** 343.

Barka, T., and P. J. Anderson. 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* **10:** 741–753.

Blitz, R. 1965. The clearance of foreign material from the coelom of *Phascolosoma agassizii.* Ph.D. Thesis Univ. California, Berkeley. 177 pp.

Boiledieu, D., and P. Valembois. 1977. Natural cytotoxic activity of sipunculid leukocytes on allogenic and xenogenic erythrocytes. *Dev. Comp. Immunol.* **1:** 207–216.

Brown, A. C., and R. Winterbottom. 1969. The fate of thorium dioxide injected into the coelom of the sipunculid, *Golfingia capensis. J. Invertebr. Pathol.* **13:** 229–234.

Burstone, M. S. 1958. Histochemical comparison of naphthol-AS-phosphatases for the demonstration of phosphatases. *J. Nat. Cancer Inst.* **20:** 601–616.

Cantacuzène, J. 1922. Reactions d'immunite chez *Sipunculus nudus* vaccinè contre une bactèrie. *C. R. Soc. Biol. (Paris)* **87:** 259–263.

Caufield, J. B. 1957. Effects of varying the vehicle for OsO_4 in tissue fixation. *J. Biophys. Biochem. Cytol.* **3:** 827–830.

Cuénot, L. 1900. Le Phascolome. Pp. 386–422 in L. Bouton, Ed., *Zool. Descriptive des Invertebres,* Vol. 1. 0. Doin, Paris.

Cushing, J. E., and D. K. Boraker. 1975. Some specific aspects of cell-surface recognition by sipunculid coelomocytes. Pp. 35–44 in W. H. Hildemann and A. A. Benedict, Eds., *Immunologic Phylogeny,* Plenum, New York.

Cushing, J. E., N. L. Calaprice, and G. Trump. 1963. Blood group reactive substances in some marine invertebrates. *Biol. Bull.* **125:** 69–80.

Cushing, J. E., J. L. McNeely, and M. P. Tripp. 1969. Comparative immunology of sipunculid coelomic fluid. *J. Invertebr. Pathol.* **14:** 4–12.

Dybas, L. 1975. Cell within a cell or a circulating cell pair. *Nature* **257:** 790–791.

Dybas, L. 1976. A light and electron microscopic study of the ciliated urn of *Phascolosoma agassizii* (Sipunculida). *Cell Tiss. Res.* **169:** 67–75.

Evans, E. E., J. E. Cushing, and M. L. Evans. 1973. Comparative immunology: Sipunculid bactericidal responses. *Infect. Immun.* **8:** 355–359.

Evans, E. E., P. F. Weinheimer, R. T. Acton, and J. E. Cushing. 1969. Induced bactericidal response in a sipunculid worm. *Nature* **222:** 695.

Goodpasture, E. W. 1919. A peroxidase reaction with sodium nitroprusside and benzidine in blood smears and tissues. *J. Lab. Clin. Med.* **4:** 442–444.

Hayat, M. A. 1970. Principles and Techniques of Electron Microscopy. Biological Applications, Vol. 1. Van Nostrand Reinhold Co., New York. 412 pp.

Hyman, L. 1959. Pp. 610–696 in *The Invertebrates: Smaller Coelomate Groups,* Vol. 5. McGraw Hill Book Co., New York.

Johnson, P. T., and F. A. Chapman. 1970. Comparative studies on the *in vitro* response of bacteria to invertebrate body fluids. I. *Dendrostomum zostericolum,* a sipunculid worm. *J. Invertebr. Pathol.* **16:** 127–138.

Klebanoff, S. F. 1967. Iodination of bacteria. A bactericidal mechanism. *J. Exp. Med.* **126:** 1063–1079.

Krassner, S. M. 1963. Further studies on an antibacterial factor in the blood of *Phascolosoma gouldii. Biol. Bull.* **125:** 382–383.

Krassner, S. M., and B. Florey. 1970. Antibacterial factors in the sipunculid worms *Golfingia gouldii* and *Dendrostomum pyroides. J. Invertebr. Pathol.* **16:** 331–338.

Lillie, R. D. 1969. H. J. Conn's Biological Stains. A Handbook on the Nature and Uses of Dyes

Employed in the Biological Laboratory, 8th Edtn. Williams and Wilkins Co., Baltimore. 498 pp.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9:** 409–414.

Malamed, S. 1963. Use of a microcentrifuge for preparation of isolated mitochondria and cell suspensions for electron microscopy. *J. Cell Biol.* **18:** 696–700.

Nicosia, S. 1979. Lectin-induced mucus release in the urn cell complex of the marine invertebrate *Sipunculus nudus* (Linnaeus). *Science* **206:** 698–700.

Ohuye, T., O. Ochi, and I. Miyata. 1961. On the morphogenesis and histochemistry of the "urn" found in the coelomic fluid of a sipunculid, *Phascolosoma scolops. Mem. Ehime Univ. Sect. II (Nat. Sci.) Ser. B (Biol.)* **4:** 145–151.

Rabin, H., and F. B. Bang. 1964. *In vitro* studies of the antibacterial activity of *Golfingia gouldii* (Pourtalès) coelomic fluid. *J. Insect Pathol.* **6:** 457–465.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain for electron microscopy. *J. Cell Biol.* **17:** 208–212.

Towle, A. 1962. Physiological changes in *Phascolosoma agassizii* Keferstein during the course of an annual reproductive cycle. Ph.D. Thesis, Stanford Univ. 163 pp.

Triplett, E. L., J. E. Cushing, and G. L. Durall. 1958. Observations on some immune reactions of the sipunculid worm *Dendrostomum zostericolum. Amer. Nat.* **92:** 287–293.

Trump, B. F., E. A. Smuckler, and E. P. Benditt. 1961. A method for staining epoxy sections for light microscopy. *J. Ultrastr. Res.* **5:** 343–348.

Valembois, P., and D. Boiledieu. 1980. Fine structure and functions of haemerythrocytes and leucocytes of *Sipunculus nudus. J. Morphol.* **163:** 69–77.

Volkonsky, M. 1933. Digestion intracellulaire et accumulation des colorants acids. Ètude cytologique des cellules sanguines des sipunculides. *Biol. Bull.* (France et Belgium) **67:** 135–199.

Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4:** 475–478.

Weinheimer, P. F., R. T. Acton, J. E. Cushing, and E. E. Evans. 1970. Reactions of sipunculid coelomic fluid with erythrocytes. *Life Sci.* **9:** 145–152.

Weiss, L. 1977. Blood. Pp. 433–457 in Weiss, L., and R. O. Greep, Eds., *Histology*. Blakiston Div., McGraw-Hill, Inc., New York.

Yam, L. T., C. Y. Li, and W. H. Crosby. 1971. Cytochemical identification of monocytes and granulocytes. *Am. J. Clin. Pathol.* **55:** 283–290.

Reference: *Biol. Bull.* **161:** 115–125. (August, 1981)

AMESON MICHAELIS (MICROSPORIDA) IN THE BLUE CRAB, *CALLINECTES SAPIDUS*: PARASITE-INDUCED ALTERATIONS IN THE BIOCHEMICAL COMPOSITION OF HOST TISSUES

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ABSTRACT

Ameson michaelis' interaction with its blue crab host significantly altered the biochemical constituents of host tissues. Modifications in skeletal muscle protein and carbohydrate metabolism were reflected in substantial variations in hemolymph composition. Blood osmolality, Cl^-, and Na^+ ion levels decreased with heavy parasitic invasion, while K^+ ion and ninhydrin positive substances (NPS) levels increased in both light and heavy infections. Microsporidiosis resulted in a general increase in all hemolymph free amino acids detected except glutamic acid.

Effects of *A. michaelis* sporogenesis were observed by comparing the biochemical composition of thoracic and cheliped skeletal musculature. Protein and carbohydrate levels were lower in infected thoracic muscle. The opposite trend was observed for tissue free-amino-acids (ninhydrin-positive substances). The concentration of nine of the sixteen amino acids detected remained unchanged with infection. Skeletal muscle glutamate, proline, glycine, alanine, and arginine levels declined, while taurine and tyrosine levels increased.

Lactic acid accumulated in the hemolymph, thoracic muscle, and hepatopancreas of parasitized blue crabs. Lactate concentrations reached six to seven times their normal levels in hemolymph and skeletal muscle, and four times the control value in the hepatopancreas. Blood glucose levels declined during the terminal stages of microsporidan infection.

INTRODUCTION

The Microsporidae are a large group of highly specialized obligate intracellular protozoan parasites. Although they are best known as parasites of arthropods and fish, microsporidans are the etiological agents of various pathologies in amphibians, reptiles, birds, and a variety of mammals, including man (see Sinderman, 1970; Weiser, 1976; Canning, 1977). Despite these parasites' wide distribution in nature and their devastating effects on a variety of animals, relatively little is known about their physiological characteristics and the infections they produce.

Disturbances in the biochemical composition of tissues infected by intracellular parasites are of interest because such infections often significantly alter the electrolyte, carbohydrate, protein, and free-amino-acid pools of host cells (see von Brand, 1973). Among parasitic protozoans, the microsporidan *Ameson michaelis* (=*Nosema michaelis*) massively invades host cells, proceeds rapidly through sporogenesis in host muscle, and inflicts widespread tissue damage. *A. michaelis* sporogenesis takes place in blue crab sarcoplasm. Ater entering myofibrils, schizonts

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differentiate into sporonts, and then multiply into sporoblasts (Weidner, 1970). The massive numbers of sporoblast colonies in the sarcoplasm result in the disorientation and eventual loss of the highly organized myofibrillar structure of the host muscle (Weidner, 1970). As the infection proceeds to its terminal stages, parasite spores largely replace the host skeletal musculature (up to 10^9 parasites/g infected muscle).

The present study characterizes the physiological consequences of microsporidan infection in blue crab skeletal muscle, hemolymph, and hepatopancreas tissues.

MATERIALS AND METHODS

Adult intermolt blue crabs (*Callinectes sapidus*), uninfected and infected with *Ameson michaelis*, were collected from Lake Pontchartrain near Irish Bayou, Louisiana, during August and September, 1978 and 1979. Animals were transported to the laboratory over ice and then killed immediately. Small sections of thoracic skeletal muscle tissue were examined microscopically to determine the extent of parasite development in individual animals. Blue crabs exhibiting $\leq 10^3$ parasites/g tissue were considered lightly infected; $> 10^3$ parasites/g tissue was considered a heavy infection.

Hemolymph analyses

Osmolality and ion analyses: The cuticle at the base of the pereiopod (swimming leg) was blotted dry and a 300–400 μl sample of hemolymph was withdrawn into snap-cap microcentrifuge tubes using unheparinized capillary tubes. Blood samples were centrifuged immediately at 10,000 *g* for 4 min to remove any particulate material. The osmolality of hemolymph and seawater samples was determined with a WESCOR Vapor Pressure Osmometer. Hemolymph samples were diluted with deionized water for ion analyses. Chloride ion concentrations were determined with an Aminco Chloridometer. Sodium and potassium levels were determined with a Coleman Flame Photometer.

Ninhydrin positive substances: Additional hemolymph samples were collected to determine ninhydrin positive substances (NPS). Protein was precipitated from the hemolymph by diluting each sample with 5,sulfosalicylic acid to a final concentration of 10%. Following centrifugation (10,000 *g*; 4 min), the NPS level of the supernatant was determined colorimetrically by the method of Rosen (1957). Leucine was used as the ninhydrin standard. Amino acid composition of the protein-free supernatant was determined with a Beckman Amino Acid Analyzer, Model 119.

Glucose analyses: Hemolymph samples (0.2 ml HL in 2.0 ml distilled water) were deproteinized by adding 1.0 ml 0.3 *N* $Ba(OH)_2$ and 1.0 ml 5% $ZnSO_4 \cdot 7H_2O$. Following centrifugation (10,000 *g*; 4 min), the glucose concentration of the protein-free supernatant was quantified colorimetrically by the glucose oxidase-peroxidase method (Sigma Chemical Co., Kit No. 510).

Lactate analyses: Immediately after they were collected, 0.2 ml hemolymph samples were transferred into microcentrifuge tubes containing 0.4 ml cold 8% perchloric acid. The tubes were agitated and returned to an ice bath for 5 min to assure complete precipitation of blood proteins. The mixture was subsequently centrifuged (10,000 *g*; 15 min) and the lactate concentration of the protein-free supernatant was quantified enzymatically (Sigma Chemical Co., Kit No. 826–UV).

Skeletal muscle and hepatopancreas analyses

Protein, NPS, and carbohydrate concentrations: Skeletal muscle tissue was removed from the thoracic region and the pereiopods (including the chela, walking legs, and paddles). Dissected tissue was flash frozen over dry ice, lyophilized to dryness, and ground to a uniform particle size in a Thomas-Wiley tissue grinder. Ground tissue was stored frozen (−20°C) in 1-dram glass vials with cork stoppers. When it was needed, ground tissue was removed from the freezer and warmed to room temperature in a dessicator to prevent water adsorption. Protein was extracted by refluxing 10 mg of the ground tissue in 1 *N* NaOH for 30 min at 100°C. The protein content of the tissue extracts was determined by the method of Lowry *et al.* (1951). Bovine serum albumin was used as the reference standard. Additional portions of the NaOH extracts were analyzed for carbohydrate concentration (Montgomery, 1957), using glycogen as the standard. Hepatopancreas samples were similarly prepared and analyzed for their protein and carbohydrate content.

To measure NPS in tissue, 10 mg of ground skeletal muscle tissue was incubated in 10% 5,sulfosalicylic acid (48 h; 22°C) to precipitate proteins and permit leaching of free amino acids from the tissue. Samples were then centrifuged (10,000 *g*; 15 min) and supernatant NPS was determined (Rosen, 1957), using leucine as the standard. Amino acid composition of the NPS digest was determined with a Beckman Amino Acid Analyzer, Model 119.

Lactate concentrations: Samples of thoracic skeletal muscle and hepatopancreas were removed, flash frozen over dry ice, and weighed. Individual samples were homogenized in 10 times their weight of cold 8% perchloric acid. Homogenates were transferred to microcentrifuge tubes and returned to an ice bath for 5 min to allow precipitation of tissue proteins. Samples were centrifuged (10,000 *g*; 15 min) and the lactate concentration of the protein-free supernatant was quantified enzymatically (Sigma Chemical Co., Kit No. 826-UV).

RESULTS

Hemolymph profile

Salinity at the time crabs were collected was 2–6% S, water temperature varied from 29–34°C, and the dissolved oxygen content ranged between 5–7 mg/l. Water under these temperature and salinity conditions was 70–95% saturated with dissolved oxygen.

During both years, *A. michaelis* infection markedly altered blue crab hemolymph composition. Blood osmolality and Cl^- and Na^+ ion levels decreased with infection, while K^+ ion and ninhydrin positive substances (NPS) levels increased (Table I). Light infection produced little change in hemolymph osmolality, Na^+, and Cl^- ion concentrations ($P > 0.05$), whereas blood K^+ values were 25% (~1.5–2.0 m*M*/1) higher, and NPS levels were nearly twice (or approximately 3–4 m*M*/l higher than) those of uninfected animals ($P < 0.05$).

A heavy parasite burden was accompanied by significant modifications in all blood constituents assayed ($P < 0.01$). Hemolymph osmolality decreased by 10% (~60–70 mOsm/kg water), and Cl^- and N^+ ion values each declined by 15% (approximately 50 m*M*/l) of their control levels. K^+ ion concentrations were 60% or nearly 5 m*M*/l higher, and NPS values 2.5–3 times or approximately 8 m*M*/l higher, than those of control animals.

The amino acid composition of hemolymph from infected blue crabs was substantially different from that of controls (Table II). *A. michaelis* infection increased

TABLE I

Effects of microsporidan infection on the hemolymph composition of blue crabs. Values are for blue crabs collected from Lake Pontchartrain, Louisiana, during the summer of 1978 (top line of each entry) and 1979 (bottom line of each entry). Salinity at the time of collection was 2–4‰ S (1978) and 2–6‰ S (1979). All hemolymph values are the mean ± 95% confidence interval; sample size in parentheses.

		Infected	
	Normal	Light	Heavy
Osmolality (mOsm/kg H_2O)	663.3 ± 17.5 (10) 671.6 ± 21.5 (5)	641.3 ± 6.7 (10) 639.5 ± 10.1 (5)	594.2 ± 10.4 (10) 594.3 ± 9.9 (5)
Cl^- m*M*/l	327.7 ± 8.1 (10) 328.0 ± 1.7 (5)	314.5 ± 13.6 (10) 317.0 ± 5.4 (5)	278.5 ± 8.6 (10) 275.0 ± 10.5 (5)
Na^+ m*M*/l	304.7 ± 8.6 (10) 304.3 ± 6.8 (5)	290.5 ± 5.9 (10) 294.7 ± 6.8 (5)	259.0 ± 13.0 (10) 261.0 ± 12.5 (5)
K^+ m*M*/l	7.6 ± 0.8 (10) 8.2 ± 0.5 (5)	9.7 ± 0.4 (10) 9.9 ± 0.5 (5)	12.2 ± 1.5 (10) 13.3 ± 1.1 (5)
NPS m*M*/l	4.1 ± 0.8 (10) 5.4 ± 0.6 (5)	8.3 ± 0.6 (10) 8.3 ± 1.1 (5)	12.4 ± 1.9 (10) 12.6 ± 2.4 (5)

the hemolymph concentration of ammonia and seven of the nine amino acids detected. Infected animals exhibited a 50% increase in hemolymph glycine concentration, a two- or three-fold increase in arginine, aspartic acid, taurine, threonine, and NH_3 levels, and a seven- or eight-fold increase in alanine and proline concentrations. Glutamic acid decreased 60%, and was the only amino acid to decline in concentration in infected animals.

Skeletal muscle profile

Microsporidan infection significantly changed host concentrations of muscle protein, free amino acids (NPS), and carbohydrates. However, since the level of these biochemical constituents varied widely among different blue crabs, data were

TABLE II

Amino acid composition of normal and infected blue crab hemolymph. All amino acid values are the mean ± 95% confidence interval; sample size in parentheses. Significance refers to t *test comparisons between normal and infected values. NS means not significant at* $P < 0.05$ *level.*

μmoles/ml	Normal (N = 4)	Infected (N = 6)	Significance
tau	0.380 ± 0.160	1.060 ± 0.264	$P < 0.01$
asp	0.156 ± 0.005	0.357 ± 0.079	$P < 0.05$
thr	0.027 ± 0.011	0.087 ± 0.021	$P < 0.05$
ser	0.084 ± 0.014	0.118 ± 0.041	NS
glu	0.200 ± 0.013	0.117 ± 0.022	$P < 0.01$
pro	0.071 ± 0.010	0.544 ± 0.129	$P < 0.01$
gly	0.767 ± 0.108	1.176 ± 0.293	$P < 0.05$
ala	0.074 ± 0.008	0.475 ± 0.141	$P < 0.01$
arg	0.162 ± 0.018	0.518 ± 0.049	$P < 0.01$
NH_3	3.235 ± 0.681	5.947 ± 0.566	$P < 0.01$

analyzed as the percent difference between the thoracic and cheliped muscle value for each constituent in single animals. Comparing thoracic and cheliped muscle values maximized the detection of infection-related changes in biochemical composition, because the thoracic region is the first and most heavily infected of the crab skeletal musculature, while the claw tissue, apparently secondarily infected, often contains few parasites until the terminal stages of the infection.

Skeletal muscle protein varied between 600–750 μg/mg dry tissue in all animals sampled. Light and heavy infections resulted in significantly lower thoracic than cheliped muscle protein (Table III). Skeletal muscle free-amino-acid levels ranged from 0.8–1.3 μmoles/mg dry tissue. In infected animals, thoracic muscle NPS concentrations were significantly higher than cheliped levels (Table III). Heavy infections produced substantially greater alterations in the protein and NPS ratio of thoracic and cheliped muscle than did lighter parasite burdens ($P < 0.01$).

The amino acid composition of infected thoracic muscle differed from that of uninfected tissue (Table IV). Infected thoracic muscle contained reduced quantities of alanine, arginine, glutamic acid, glycine, and proline. Arginine levels dropped ~65%, alanine and proline values declined ~50%, and glutamic acid and glycine decreased approximately 30% in concentration. Infected muscle taurine and tyrosine values were twice those of normal animals. Muscle aspartic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, and valine concentrations remained the same as their control values ($P > 0.05$). Tissue NH_3 levels also did not change with infection ($P > 0.05$).

Skeletal muscle carbohydrate concentration varied from 20–35 μg/mg dry tissue in all animal groups. Both light and heavy infections produced significantly lower thoracic than cheliped muscle carbohydrate levels ($P < 0.01$) (Table III). The conditions in infected animals also differed statistically from the control condition ($P < 0.01$).

Parasitic infection produced no significant differences in hepatopancreas carbohydrate (from 15.9 ± 0.5 to 15.2 ± 0.9 μg/mg dry tissue, $P > 0.05$) or protein (from 250.0 ± 11.7 to 223.0 ± 10.4 μg/mg dry tissue, $P > 0.05$) levels.

Lactate levels

Microsporidan infection substantially increased lactic acid levels in hemolymph, skeletal muscle, and hepatopancreas host tissues (Table V). Hemolymph samples had the lowest control lactic-acid levels. Light infections produced a fourfold in-

TABLE III

Effect of microsporidan infection on the skeletal muscle composition of blue crabs. Data are the percent difference between thoracic and cheliped muscle levels of protein, free amino acid (NPS), and carbohydrates. Arrows indicate whether values for thoracic muscle were higher (↑) or lower (↓) than those of cheliped muscle. All values are the mean ± 95% confidence interval; sample size in parentheses.

		Infected	
	Normal (N = 6)	Light (N = 10)	Heavy (N = 6)
Protein	↑0.97 ± 0.24	↓5.98 ± 1.18	↓12.45 ± 0.65
NPS	↑2.60 ± 0.93	↑11.49 ± 3.46	↑27.03 ± 5.45
Carbohydrate	↑6.00 ± 0.71	↓4.65 ± 1.21	↓18.07 ± 3.04

Table IV

Amino acid profile of thoracic skeletal muscle from normal and infected blue crabs. All amino acid values are the mean ± 95% confidence interval; sample size in parentheses. Significance refers to t *test comparisons between values. NS means not significant at the* P < *0.05 level.*

μmoles/mg	Normal (N = 4)	Infected (N = 6)	Significance
tau	0.096 ± 0.019	0.193 ± 0.038	$P < 0.05$
asp	0.029 ± 0.003	0.029 ± 0.003	NS
ser	0.043 ± 0.008	0.044 ± 0.015	NS
glu	0.095 ± 0.011	0.061 ± 0.012	$P < 0.05$
pro	0.189 ± 0.027	0.095 ± 0.034	$P < 0.05$
gly	0.508 ± 0.049	0.352 ± 0.053	$P < 0.05$
ala	0.192 ± 0.032	0.106 ± 0.027	$P < 0.05$
val	0.015 ± 0.001	0.014 ± 0.002	NS
met	0.011 ± 0.004	0.010 ± 0.004	NS
ile	0.009 ± 0.004	0.010 ± 0.004	NS
leu	0.030 ± 0.023	0.016 ± 0.009	NS
tyr	0.011 ± 0.001	0.022 ± 0.003	$P < 0.05$
phe	0.008 ± 0.001	0.009 ± 0.004	NS
his	0.017 ± 0.004	0.015 ± 0.005	NS
lys	0.021 ± 0.003	0.021 ± 0.007	NS
arg	0.319 ± 0.037	0.099 ± 0.030	$P < 0.01$
NH_3	0.116 ± 0.022	0.130 ± 0.028	NS

crease and heavy infections generated a sevenfold increase in crab hemolymph lactate concentration ($P < 0.01$). Light parasite burdens doubled-thoracic muscle lactate ($P < 0.01$), whereas heavy infections yielded a sixfold increase in muscle lactate ($P < 0.01$). Control lactate values were higher for the hepatopancreas than for either of the other normal tissues. A 65% elevation of hepatopancreas lactate concentrations accompanied light infections ($P < 0.05$), while heavy infections produced a 4.5-fold increase in normal lactate levels ($P < 0.01$).

Blue crabs also evidenced hypoglycemia in the terminal stages of *A. michaelis* infection. Hemolymph glucose levels declined from a control value of 66.3 ± 2.8 mg/100 ml to 51.1 ± 3.5 mg/100 ml. This was significant difference ($P < 0.01$) between normal and infected animals.

DISCUSSION

Little of the considerable literature pertaining to the Microsporida deals with physiological changes in host animals as a result of microsporidan infection. During

Table V

Lactate concentration in the hemolymph, thoracic skeletal muscle and hepatopancreas of blue crabs. All values are the mean ± the 95% confidence interval; sample size in parentheses.

	Normal (N = 6)	Infected Light (N = 6)	Infected Heavy (N = 6)
Hemolymph mg/ml	0.110 ± 0.050	0.441 ± 0.036	0.722 ± 0.032
Thoracic muscle mg/g	0.228 ± 0.051	0.548 ± 0.022	1.237 ± 0.276
Hepatopancreas mg/g	0.314 ± 0.069	0.512 ± 0.062	1.465 ± 0.322

infection, the host cell environment may influence the metabolic activities of an intracellular parasite. In the present study, we have shown that the interactions of the microsporidan parasite *Ameson michaelis* with its blue crab host, *Callinectes sapidus*, result in thoracic skeletal muscle alterations that are ultimately reflected in biochemical changes in the host blood.

The estuarine environment of the blue crab experiences rapid fluctuations in osmotic and ionic composition. *Callinectes sapidus* has considerable capacity for osmotic and ionic regulation over widely fluctuating salinity regimens (Findley and Stickle, 1978). This capacity for water and salt regulation varies with a number of exogenous and endogenous factors, among the latter parasitism. *A. michaelis* appears to infect blue crabs most readily in areas of low salinity, high temperature, and reduced oxygen (personal observation). The same combination of factors also stresses the crabs' regulatory abilities (Kinne, 1971).

The hemolymph composition of control crabs used in this study was consistent with that previously described for blue crabs maintained at extremely low salinities (Mangum and Amende, 1972; Cameron, 1978). As a consequence of parasitic infection, hemolymph osmotic and ionic composition changed significantly. Blood osmolality, Cl^-, and Na^+ ion concentrations decreased with infection, while K^+ ion and free amino acid (NPS) levels increased. This may result in part from the release of ions and free amino acids accompanying host cell lysis. However, cell lysis cannot account for the drop in blood osmolality during heavy infection, because the extracellular and intracellular fluid compartments are isosmotic. Blood composition was significantly changed primarily during heavy microsporidan infection. Severe parasite invasion results in the production of large numbers of mature spores and concomitant disruption of host skeletal muscle tissue (Weidner, 1970). Animals supporting light infections (with proportionally larger numbers of young sporoblast stages present) did not have substantially altered hemolymph profiles. Presumably, during light infection, blue crabs are able to accomodate initial host-cell lysis. However, their regulatory abilities may become inadequate to handle the extensive cell damage that occurs during the terminal stages of infection.

In contrast to our data, Vivarès and Cuq (1981) observed no change in hemolymph ion content of crabs (*Carcinus mediterraneus*) parasitized by the microsporidan *Thelohania maenadis*. However, the differing environmental regimens to which the two species of crab were exposed (2–6‰ S; 29–34°C for *Callinectes sapidus* vs. 33‰ S; 18°C for *Carcinus mediterraneus*) may explain these conflicting results, and consequently, the relative importance of the host blood ion composition during parasitic infection. *C. sapidus* maintained at extremely low salinities must regulate its hemolymph hyperosmotic and hyperionic to the dilute media. Elevated water temperature and reduced oxygen further aggravates this situation. Conversely, *C. mediterraneus* held at higher salinities (generally isosmotic and isionic to the crab blood) does not have water and salt regulation as limiting factors for survival.

A. michaelis infections also substantially altered the hemolymph free-amino-acid profile. In salinities of 16.5–21.0‰ S, glycine, taurine, alanine, proline, and arginine constitute 72–90% of the total free amino acids in the serum of mature *Callinectes sapidus* (Lynch and Webb, 1973a). At extremely low salinities (2–4‰ S), we detected only nine amino acids and ammonia in appreciable quantities. The relative concentrations of proline and alanine appeared to be reduced, while the levels of glutamic and aspartic acids were proportionally increased.

In the blue crab, microsporidan infection contributed to a general elevation of all blood free amino acids except glutamic acid. These increases may be primarily drived from host muscle proteolysis, but parasite invasion may also modify the permeability of muscle cell membranes, so that intracellular amino acids leak to the extracellular spaces. Such a mechanism operates in osmotic and volume regulation in euryhaline animals (Fugelli, 1967; Vincent-Marique and Gilles, 1970). Accumulation of amino acids in the body fluids of infected animals may indicate that their capacity to hydrolyze host cell protein is greater than their ability to incorporate the resultant amino acids into parasite protein (Moulder, 1962). Therefore, the decline we observed in hemolymph glutamic acid may indicate its uptake by developing parasites. Similarly, parasites may use those amino acids which increase only moderately in infected animals (*i.e.*, glycine, aspartic acid, and serine). In fact, acid hydrolysates of *Ameson michaelis* spores indicate large quantities of glutamic acid, glycine, aspartic acid, and serine, in addition to significant amounts of tyrosine (Weidner and Blakeney, unpublished data).

Only two studies deal with host blood amino acids during microsporidiosis (Wang and Moeller, 1970; Vivarès *et al.*, 1980). *Nosema apis*-infected honey bees have less hemolymph amino acids than their non-infected counterparts. Wang and Moeller (1970) suggested this decrease reflects the parasite's utilization of the host blood free-amino-acid pool. At first glance, our data, describing increased levels free amino acids in plasma, appear inconsistent with those of Wang and Moeller. However, this discrepancy is probably attributable to the use of animals supporting different stages of microsporidan infection. Five- to ten-day-old infected honey bees contain proportionally larger numbers of younger, more metabolically active vegetative and early spore-forming stages than do blue crabs sustaining terminal infections, represented by the abundance of mature spores. Additionally, honey bees, as insects, have a larger hemolymph free-amino-acid pool (~28–65 m*M*/l), and consequently a more substantial amino acid source for developing parasites, than do blue crabs, which have lower blood amino-acid levels (~4–5 m*M*/l at 2–6‰ S). Vivarès *et al.* (1980) monitored the effect of *Thelohania maenadis* infection on the hemolymph amino acid composition of *Carcinus mediterraneus*. Although parasitism did not substantially change the total free-amino-acid level, the concentration of several amino acids increased moderately (including taurine, cysteic acid, aspartic acid, threonine, serine, glutamic acid, alanine, methionine, histidine, and arginine), while proline and glutamine levels decreased.

Ameson michaelis initially invades skeletal muscle in the thoracic region, near the crab midgut submucosa, which supports the parasite's early vegetative development. As sporogenesis proceeds, other skeletal muscle masses (*i.e.*, those of the cheliped and other pereiopods) are infected (Weidner, 1970). Comparison of thoracic and cheliped muscle composition reflects the effects of sporogenesis. Significant decreases in tissue protein and concomitant increases in free-amino-acid levels may result from substantial muscle proteolysis accompanying terminal parasite development. Additionally, modified membrane permeability may change the relative abundance of muscle, and consequently, hemolymph free-amino-acids (Schoffeniels, 1976). Reduced muscle carbohydrate reserves (*i.e.*, glycogen) may result from increased metabolic demands of host muscle and/or developing parasites.

Low salinity significantly reduced the crab-muscle free-amino-acid pool (~0.8–1.3 μmoles/mg dry tissue, 2–4‰ S, present study, vs. ~28 μmoles/100 mg wet weight, 50% SW and ~42 μmoles/100 mg wet weight, 100% SW, Schoffeniels, 1976). Blue crabs transferred from full-strength to 50% seawater display significant decreases in virtually all muscle free amino acids (Schoffeniels, 1976). At even lower salinities, we observed further reductions in amino acid levels, and in particular, in alanine, glycine, proline, and taurine concentrations. In parasitized thoracic skeletal muscle, the concentration of nine of the sixteen amino acids detected was not significantly different. Glutamic acid, proline, glycine, alanine, and arginine levels were lower with infection, while taurine and tyrosine values were higher. Modifications in infected-muscle free-amino-acid levels, with the exception of tyrosine, were reflected in significant changes in plasma concentration. Tyrosine was virtually absent from the hemolymph of low salinity animals but was present in substantial amounts in the acid hydrolysate of *A. michaelis* spores (Weidner and Blakeney, unpublished data). Efficient uptake of tyrosine from the muscle free-amino-acid pool by developing parasites may preclude its measurable accumulation in crab blood.

Microsporidiosis in *Carcinus mediterraneus* only slightly changed the total free-amino-acid concentration of infected skeletal muscle (Vivarès *et al.*, 1980). Increases in the level of several amino acids (taurine, cysteic acid, serine, glutamic acid, alanine, methionine, leucine, tyrosine, phenylalanine, lysine, and histidine) were offset by reductions in other amino acids (threonine, proline, glycine, and arginine). Direct comparison of our results with those of Vivarès *et al.* (1980) is limited by the lack of any statistical treatment of the amino acid data in the latter study. In addition, different environmental salinity-temperature conditions may have changed the relative importance of the host amino-acid pool.

Parasitic infection may result in host tissues being deprived of oxygen (von Brand, 1973). Oxygen insufficiency can result from the increased metabolic demands of host tissues and/or developing parasites, or an impaired oxygen-delivery system. Under these conditions, anaerobic metabolism becomes increasingly important as a means of energy production (Burke, 1979). In crustaceans, glycolysis is a major functional anaerobic pathway. During hypoxia, significant quantities of pyruvate are converted to lactic acid in crustacean skeletal muscle (Dendinger and Schatzlein, 1973; Phillips *et al.*, 1977; Burke, 1979). Non-parasitized blue crabs have hemolymph and thoracic-muscle lactic-acid concentrations similar to those of other crustaceans (Dendinger and Schatzlein, 1973; Burke, 1979). However, lactic-acid concentrations in blue crab hepatopancreas are significantly higher than in other crabs (Dendinger and Schatzlein, 1973). Elevated lactate concentrations may reflect animals collected from a stressed environment (water of low salinity, high temperature, and reduced oxygen) or may be an artifact of handling (Dendinger and Schatzlein, 1973).

A. michaelis infection produced significant lactic acid accumulation in the hemolymph, thoracic muscle, and hepatopancreas of parasitized blue crabs. Lactate concentrations reached six–seven times their normal levels in hemolymph and muscle, and four times the control value in the hepatopancreas. Since the amount of lactate was always higher in the thoracic muscle than in the hemolymph, it is reasonable to assume that the muscle is the ultimate source of lactate production (Phillips *et al.*, 1977). The hepatopancreas has both reduced activity of glycolytic enzymes and an insignificant level of *in vitro* lactate production (Dendinger and Schatzlein, 1973; Schatzlein *et al.*, 1973). Therefore, increases in hepatopancreas lactate levels probably represent the clearance of this muscle metabolite from the hemolymph.

Plasma glucose concentrations in freshly collected *Callinectes sapidus* specimens are extremely variable. Our control blood-glucose values were at the high end of the reported normal range (Dean and Vernberg, 1956a; Lynch and Webb, 1973b). Elevated glucose concentrations may be due to handling stress (Telford, 1968), asphyxia (resulting from holding animals out of water) (Lynch and Webb, 1973b), or high temperatures (Dean and Vernberg, 1965b). Parasitized blue crabs had reduced blood glucose levels during the terminal stages of microsporidan infection (a terminal hypoglycemia). Reduced hemolymph glucose may result from increased metabolic demands of host cells and/or developing parasites.

Lactic acid accumulates in the blood of protozoan-infected animals (von Brand, 1973). However, in whole animal studies, it is difficult to ascertain whether elevated lactate concentrations result from parasite production or host cell metabolism. The relative importance of host cell and parasite carbohydrate metabolism in accumulation of lactate by microsporidan-infected blue crabs cannot be determined directly from the present study.

Microsporidan infection contributed to significant alterations in the biochemical constituents of blue crab host tissues. Modifications in skeletal muscle protein and carbohydrate metabolism were ultimately reflected in substantial changes in hemolymph composition. Characterizing the blue crab host cell environment during *Ameson michaelis* infection provides critical background information for future metabolic studies of isolated, extracellularly maintained microsporidan parasites.

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LITERATURE CITED

Burke, E. M. 1979. Aerobic and anaerobic metabolism during activity and hypoxia in two species of intertidal crabs. *Biol. Bull.* **156:** 157–168.

Cameron, J. N. 1978. NaCl balance in the blue crab, *Callinectes sapidus*, in fresh water. *J. Comp. Physiol.* **123:** 127–135.

Canning, E. U. 1977. Microsporida. Pp. 155–196 in J. P. Kreier, Ed., *Parasitic protozoa*, Volume IV. Academic Press, New York.

Dean, J. M., and F. J. Vernberg. 1965a. Variations in the blood glucose of Crustacea. *Comp. Biochem. Physiol.* **14:** 29–34.

Dean, J. M., and F. J. Vernberg. 1965b. Effects of temperature acclimation on some aspects of carbohydrate metabolism in decapod Crustacea. *Biol. Bull.* **129:** 87–94.

Dendinger, J. E., and F. C. Schatzlein. 1973. Carbohydrate metabolism in the striped shore crab, *Pachygrapsus crassipes*. II. Glycolytic rate of muscle, gill and hepatopancreas. *Comp. Biochem. Physiol.* **45B:** 699–708.

Findley, A. M., and W. B. Stickle. 1978. Effects of salinity fluctuation on hemolymph composition of the blue crab *Callinectes sapidus*. *Mar. Biol.* **46:** 9–15.

Fugelli, K. 1967. Regulation of cell volume in flounder (*Pleuronectes flesus*) erythrocytes accompanying a decrease in plasma osmolality. *Comp. Biochem. Physiol.* **22:** 253–260.

Kinne, O. 1971. Salinity: animals—invertebrates. Pp. 821–995 in O. Kinne, Ed., *Marine biology*, Vol. 1, Part 2. Wiley Interscience, New York.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

Lynch, M. P., and K. L. Webb. 1973a. Variations in serum constituents of the blue crab, *Callinectes sapidus*: free amino acids and total ninhydrin positive substances. *Comp. Biochem. Physiol.* **45B:** 407–418.

Lynch, M. P., and K. L. Webb. 1973b. Variations in serum constituents of the blue crab, *Callinectes sapidus*: Glucose. *Comp. Biochem. Physiol.* **45A:** 127–139.

Mangum, C. P., and L. M. Amende. 1972. Blood osmotic concentration of blue crabs (*Callinectes sapidus* Rathbun) found in fresh water. *Chesapeake Sci.* **13:** 318–320.

Montgomery, R. 1957. Determination of glycogen. *Arch. Biochem. Biophys.* **67:** 378–386.

Moulder, J. 1962. *The biochemistry of intracellular parasitism.* University of Chicago Press, Chicago.

Phillips, J. W., R. J. W. McKinney, F. J. R. Hird, and D. L. Macmillan. 1977. Lactic acid formation in crustaceans and the liver function of the midgut gland questioned. *Comp. Biochem. Physiol.* **56B:** 427–433.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.* **67:** 10–15.

Schatzlein, F. C., H. M. Carpenter, M. R. Rodgers, and J. L. Sutko. 1973. Carbohydrate metabolism in the striped shore crab, *Pachygrapsus crassipes.* I. The glycolytic enzymes of gill, hepatopancreas, heart and leg muscles. *Comp. Biochem. Physiol.* **45B:** 393–405.

Schoffeniels, E. 1976. Biochemical approaches to osmoregulatory processes in Crustacea. Pp. 107–124 in P. S. Davies, Ed., *Perspectives in experimental biology*, Vol. 1. Pergamon Press, New York.

Sinderman, C. J. 1970. *Principal diseases of marine fish and shellfish.* Academic Press, New York.

Telford, M. 1968. The effects of stress on blood sugar composition of the lobster, *Homarus americanus. Can. J. Zool.* **46:** 819–826.

Vincent-Marique, C., and R. Gilles. 1970. Modification of the amino acid pool in blood and muscle of *Eriocheir sinensis* during osmotic stress. *Comp. Biochem. Physiol.* **35:** 479–485.

Vivarès, C. P., J.-L. Cuq, H. J. Ceccaldi, and P. J. Richard. 1980. Influence d'une microsporidiose sur les acides aminés libres de *Carcinus mediterraneus* Czerniavsky, 1884 soumis à diverses salinités et a des valeurs extrêmes de température. *J. Exp. Mar. Biol. Ecol.* **43:** 207–220.

Vivarès, C. P., and J.-L. Cuq. 1981. Physiological and metabolic variations in *Carcinus mediterraneus* (Crustacea:Decapoda) parasitized by *Thelohania maenadis* (Microspora:Microsporida): An ecophysiopathological approach. *J. Invertebr. Pathol.* **37:** 38–46.

von Brand, T. 1973. *Biochemistry of parasites.* Academic Press, New York.

Wang, D. I., and F. E. Moeller. 1970. Comparison of the free amino acid composition in the hemolymph of healthy and *Nosema*-infected female honey bees. *J. Invertebr. Pathol.* **15:** 202–206.

Weidner, E. 1970. Ultrastructural study of microsporidan development. *Z. Zellforsch.* **105:** 33–54.

Weiser, J. 1976. Microsporidia in invertebrates: Host-parasite relations at the organismal level. Pp. 163–201 in L. A. Bulla, Jr. and T. C. Cheng, Eds., *Comparative pathobiology*, Vol. 1. Plenum Press, New York.

Reference: *Biol. Bull.* **161:** 126–140. (August, 1981)

FEEDING AND PHARMACOLOGICAL PROPERTIES OF *ANISODORIS NOBILIS* INDIVIDUALS (MOLLUSCA; NUDIBRANCHIA) SELECTING DIFFERENT NOXIOUS SPONGES PLUS DETRITUS

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ABSTRACT

Individual differences in *Anisodoris nobilis* food selection were sought in the natural environment, to determine why their digestive glands differed pharmacologically. Of 73 stationary *A. nobilis* specimens turned over in the initial survey, 84% of those on sponges and 65% of those on detritus had their mouths everted; no others were feeding. Microacoustic observations showed that an individual stops on a sponge, everts its proboscis for a long period, then rasps the sponge. Field studies of individual diets were made without disturbing the animals, by tabulating the foods on which each nudibranch was found to be stationary.

Twelve individual nudibranchs observed subtidally about three times daily for 8 days differed significantly in the sponge species they selected. Each individual was found stopped on detritus during roughly 50% of the observations, remaining there for ~1 day at a time. Each nudibranch stopped for <2 days on a sponge, sometimes returning to that spot during subsequent days. Among individuals transplanted to different feeding sites, each everted its mouth only when on the sponge species it had been eating at the previous site. Two months of subsequent, scattered observations showed occasional longer-term feeding differences.

Noxious histamine (present in each sponge) and doridosine (found only in the nudibranch) accounted for different pharmacological properties in bioassays of *Anisodoris* digestive gland extracts. Histamine disappeared when the nudibranchs ate detritus, which suggests that this mixed diet purges sponge compounds from the nudibranch. Extracts from edible sponges administered to nudibranchs caused fleeing, while extracts of avoided sponges caused violent contractions, increased mucus production, and even death.

INTRODUCTION

Studies on food selection and other resource utilization usually deal with pooled data on groups of individual organisms. However, adjacent and apparently similar members of the same species may select different diets in "intraspecific resource partitioning" (Kitting, 1979). Roughgarden (1972) considers theoretical aspects of conspecific individuals choosing different foods from a "continuous" resource axis. The present study uses graphic methods to analyze the behavior of individuals in relation to certain discrete resources that have pharmacological effects on the animal.

The dorid nudibranch *Anisodoris nobilis* (MacFarland, 1905) has biologically potent compounds concentrated in the digestive gland (Fuhrman *et al.*, 1980). This and other species of dorid nudibranchs feed largely on sponges (Harris, 1973),

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which themselves often contain unusual noxious compounds (*e.g.* Ciereszko, 1970; Bakus and Green, 1977; Minale, 1978). The tropical nudibranch *Phyllidia varicosa* concentrates terpenoid compounds from sponges (see Burreson *et al.*, 1975). Some anaspidean opisthobranchs, relatives of the nudibranchs, also modify such compounds (Faulkner and Ireland, 1977), and perhaps some nudibranchs do this too.

Initial pharmacological studies on *Anisodoris nobilis* from the Monterey Bay area showed that extracts of different groups of individuals had different effects on rate and amplitude of contractions of mammalian atria and smooth muscle (Fuhrman *et al.*, 1979). The present study shows that these differences in pharmacological properties reflect sponges or detritus eaten recently by individual nudibranchs, which differ in the sponges they select. The study also illustrates how a consumer's foraging patterns may minimize harmful effects of noxious foods.

Materials and Methods

Field experiments were carried out 100 m offshore from Hopkins Marine Station (36°37′N; 121°54′W) at the south end of Monterey Bay, California. The study area is a rocky subtidal habitat near 10 m depth within a large stand of giant kelp, *Macrocystis pyrifera. Anisodoris nobilis* adults measuring about 3–6 cm in length were common on the sides of large rocks up to 4 m in diameter. These rocks were separated by coarse sand. Individual nudibranchs were generally several meters apart, slow-moving, of different sizes, and with individually distinctive color markings. Therefore, I was able to recognize and observe specific individuals for over a week without artificial marking. Periodically, underwater photographs of each individual were made to confirm its identity.

Sponges eaten by the nudibranchs encrusted the substratum in thin sheets. Nudibranch feces were collected *in situ* with eye droppers, and samples of spicules were taken from sponges for use in identification. To assess nudibranch diets in the natural environment without disturbing the animals, I recorded the foods on which nudibranchs were observed to be stationary. I could rarely see if the nudibranchs were eating unless I turned the animals over, but in initial observations, 26 of the 31 *Anisodoris nobilis* found stationary on sponges were feeding (see below). Often, sponge had been excavated beneath the mouth of a stationary individual. Nudibranchs moving over sponges or detritus were never found feeding. Defecation was rarely seen, but the few successful fecal collections confirmed that the sponge species under an everted mouth had been consumed. Therefore, tabulations of foods under stationary individuals reflected their diets. Attempts to conduct feeding experiments in the laboratory were generally unsuccessful. Nudibranchs kept in aquaria with rocks bearing sponges moved about a great deal and seldom fed.

To determine the diets of *Anisodoris nobilis* individuals, I followed 12 nudibranchs on three large subtidal rocks abundantly supplied with the four types of food most commonly eaten. About 3 dives were made each day for 8 successive days during late September 1978, usually at 0800, 1200, and 1600 h. During each dive, I mapped each individual's location, measured the distance from the point where it was last seen, and noted any food on which it was sitting if the animal was stationary. From early October through February 1979, further detailed observations in the study area supplemented this routine sampling. Statistical methods used on the data appear in Sokal and Rohlf (1969).

Sponges and nudibranchs used for chemical assays were collected adjacent to the primary study area. Chemical extractions and sensitive pharmacological bioassays in which I merely assisted were carried out by Prof. Frederick Fuhrman and

Mrs. Geraldine Fuhrman of the Department of Physiology, Stanford Medical School. The assay methods, outlined in Fuhrman *et al.* (1979) and Fuhrman *et al.* (1980) make use of the spontaneously beating guinea pig heart *in vitro* (Perry, 1968) to detect concentrations of compounds such as ~0.4 μg histamine, or ~15 μg adenosine, per gram of nudibranch, sponge, or other tissues (Chang *et al.*, 1978). Logarithmic increases in dose were necessary to cause linear changes in response (Fuhrman, unpublished data).

I also conducted a bioassay on nudibranchs to detect toxic effects of extracts of various foods on them. An extract of each food (a sponge species or detritus from the habitat) was prepared by grinding 0.50 g of fresh sample in 1.0 ml of filtered seawater in a mortar. The toxic effects were assessed by observing changes in nudibranch movement and heart rate after extracts were applied. I timed sequences of heart beats by observing the heart's shadow with a 7× microscope. A focused, 7.5-volt light source illuminated each nudibranch from below its clear bowl of seawater. Only nudibranchs less than 4 cm in length were thin enough to use.

Some extracts were administered externally to nudibranchs, to approximate natural exposure of feeding animals to exudates of macerated food. Doses consisting of 0.05-ml samples of extract were perfused near the heart and gills of individuals 2–4 cm long, which were kept in 200-ml bowls of clean seawater at 12.5 ± 0.25°C. A solution of serotonin (10^{-5} *M* in filtered seawater) was used for comparison. Serotonin (5-hydroxytryptamine) is a molluscan heart neurotransmitter known to increase the rate of contraction of clam heart if applied directly to the heart at 10^{-6} *M* (Greenberg *et al.*, 1973). Filtered seawater was applied similarly as a control.

Other extracts were applied internally to nudibranch hearts *in vivo.* These extracts were diluted 1:10 with filtered seawater before use, and were injected into the pericardium. Nudibranchs 4 cm long received 0.2-ml doses and those 2 cm long received 0.05 ml, the doses representing roughly 2% of the digestive gland volume. Again, serotonin (10^{-6} *M* in this case) and filtered seawater were used for comparison. To test for the generality of the results obtained with *Anisodoris*, I also administered extracts to the more common dorid nudibranch *Doriopsilla albopunctata.*

RESULTS

Foraging behavior

In the laboratory, when *Anisodoris* stopped on a sponge, the animal remained in place for up to ~2 days. Whenever an individual remained stationary with its proboscis visible under its dome-like body, or when stationary animals were lifted slightly at intervals, the mouth region was found to be everted for long periods. Underwater microacoustic observations with a contact microphone (Meshna, Inc., P.O. Box 62, E. Lynn, Mass. 01904; also see Kitting, 1979) placed on sponges showed that radular scraping occurred less than 1% of the time in either light or darkness, even when the proboscis was everted. Sponge tissue under the nudibranch's mouth appeared partially dissolved even before it was seen or heard to be rasped, as though external digestion occurred first. The rasping that finally occurred was gentle brushing and irregular picking at the sponge, which is packed with siliceous spicules about 0.3 mm in length. Each nudibranch excavated a cavity up to about one-fifth its own body size in a sponge in about 2 days. Since this feeding involved eversion of the mouth followed by occasional rasping, eversion of the mouth was interpreted as "feeding behavior" in the observations below.

Overall, *Anisodoris* was found with its mouth everted on the following foods, listed in order of decreasing frequency: finely divided detritus adhering to the surface of encrusting algae or open rock; an orange sponge, *Axocielita* (=*Esperiopsis*) *originalis*; a crimson sponge, *Antho* (=*Isociona*) *lithophoenix*; a gray sponge, *Astylinifer arntdi*, growing on heavy lower blades of kelp, *Cystoseira osmundacea*; and a blue sponge, *Hymenamphiastra cyanocrypta*. Actual feeding damage was observed on each of the sponges. Additionally, fecal analyses showed that *Anisodoris* also ate a less common sponge, *Myxilla parasitica*. When *Anisodoris* was examined during 98 initial daytime field observations, the mouth was everted in 26 of the 31 animals stopped on sponges, in the 26 of the 40 stopped on detritus, but in none of the 25 animals traveling. The remaining two animals were stopped on clean encrusting algae, and were not feeding. Supplementary diving and laboratory observations made at night showed no noticeable differences in *Anisodoris* behavior.

Observations on the 12 or more *A. nobilis* specimens within a 100 m^2 area examined thrice daily for 8 successive days are summarized in Table I. Individual nudibranchs often remained stationary for several successive observations. The animals averaged only 9 cm net displacement between successive observations, but even this limited movement made all four common foods readily accessible to all individuals. Typically, after traveling for about a day, an individual appeared to remain in the same location and orientation on a sponge for up to about 2 days, usually with its mouth everted. Then the site would be found vacant, with a small wound in the sponge, while the nudibranch was found up to 2 m away, eating detritus or crawling. During at least 6 of 20 instances in which animals traveled but were found consistently, the individual returned to within <10 cm of its old feeding site (e.g., Fig. 1). One individual returned 2 m to its previous feeding site within 4 h. Excavated areas of sponge appeared to regenerate quickly (Fig. 1), and in no instance was a patch of sponge found to disappear with repeated feeding.

Differences in the foods on which individuals stopped

The foods used by each individual repeatedly examined in the field are compared in Table I. The only food omitted is the very sparse detritus on algae. No individual nudibranch was found on every dive, and several foraging records ended because the individuals were collected. On the average, each animal was observed stationary on food 7–8 different times. A G test showed highly significant differences among the sets of foods that different individuals occupied ($G = 121.19$; 33 df; $P \ll 0.005$). Similar differences were detected for those six animals found stationary on food in at least five observations throughout the study ($G = 36.92$; 10 df; $P < 0.005$). To help ensure independence of observations from one sampling time to the next, data taken closest to noon each day (for each individual) were also tested. This yielded a similar outcome ($G = 49.28$; 16 df; $P < 0.005$).

Since complete sequences of observations were not available for each individual, it is possible that on occasions when they were *not* seen they were away eating other foods. Therefore the data were also supplemented to bias them in favor of the null hypothesis (that foods used were independent of the individual's identity); hypothetical data were added as though each individual ate an additional food each time it disappeared and then reappeared during the sequence of observations. Even

TABLE I

Foraging records of adjacent Anisodoris nobilis *individuals on detritus* (=De), *encrusting noncalcareous algae* (=En), *crustose coralline algae* (=Cr), *erect algae* (=Er), *red* Axocielita (=R), *blue* Hymenamphiastra (=B), *gray* Astylinifer (=G), *or traveling* (=→). Dot = not observed; ? = food could not be determined.

	Material at anterior if nudibranch was stationary																	
	Date for sampling times:																	
Animal	9/25			9/26			9/27			9/28		9/29		9/30			10/1	10/2
A	→	De	·	·	→	·	·	R	De	R	G	·	·	R	R	De	R	Cr
B	De	R	R	Er	·	·	·	De	R	R	R	R	R	R	De	R	De	Cr
F	De	De	En	En	→	En	·	·	·	·	·	·	·	·	·	·	·	·
I	De	·	De	·	De	De	·	B	·	·	·	R	·	·	·	·	·	·
J	·	·	En	De	R	R	De	De	→	En	·	·	·	·	·	·	·	·
M	De	De	→	·	R	De	→	→	En	Er	R	R	R	R	De	De	R	·
Q	·	·	→	→	→	·	→	→	→	→	→	→	→	En	→	De	→	De
R	→	·	·	?	R	·	R	R	R	R	R	·	R	R	R	R	R	R
S	B	B	B	B	B	·	B	·	B	B	B	En	·	B	B	B	B	B
W	·	·	R	·	·	·	En	?	De	·	·	De	→	·	·	·	·	·
Z	·	·	Er	·	·	·	·	·	·	R	R	De	·	→	De	→	R	R
ZZ	·	→	→	→	En	→	?	?	De	De	Er	Er	En	De	·	·	De	·

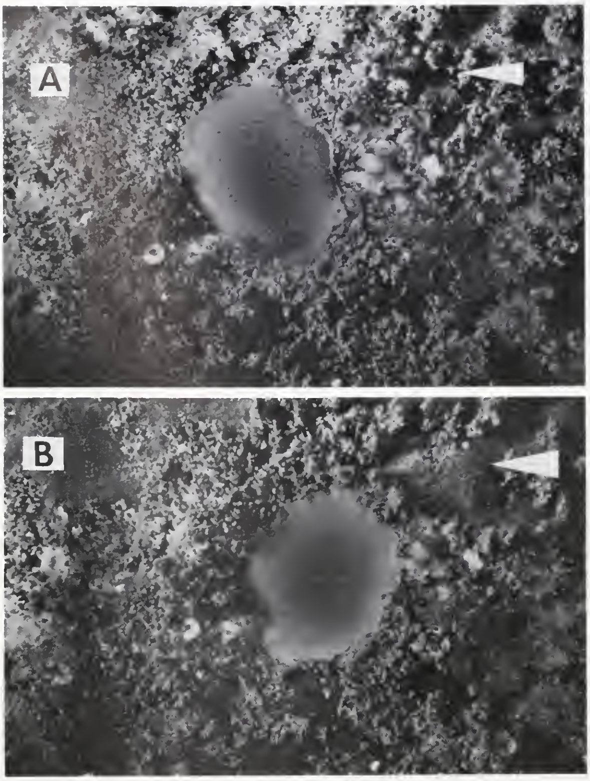

FIGURE 1. *Anisodoris nobilis* "Individual R" measuring 20 mm, down in an irregularity among its sponge food, *Antho lithophoenix*, photographed 5.5 weeks apart in the same place (also suitable for stereo viewing). (A) December 4, 1978. Retracted rhinophores are positioned toward bottom of photograph. Extensive feeding damage since October 25 is evident as upper left and upper right dark cavities (see arrow). (B) January 12, 1979. Retracted rhinophores are toward top of photograph. Feeding damage has regenerated extensively in upper left, and partially in upper right (see arrow).

under this conjectural condition, the differences among individuals remained highly significant ($G = 143.49$; 44 df; $P \ll 0.005$).

Applying data on individual foraging to a graphic model developed by Kitting (1978) enabled more detailed analysis of differences in discrete resources used by individuals. Figure 2 illustrates the frequency distributions of individual diets where the percentage of times the individual was observed to be stationary on each food resource defines its "diet" (as a resource use). Hypothetical frequency distributions were calculated from binomial expansions of the average dietary percentage represented by each food, for all individuals combined. The formula for the plot is a binomial expansion,

$$(b + a)^n = \sum_{i=0}^{n} \binom{n}{i} a^{n-i} b^i$$

where a is the average percentage of feeding on a particular food, b is the percentage of feeding on other foods ($=1 - a$), n is the average number of observations (about seven) in which an individual was found stationary on food, and the coefficient

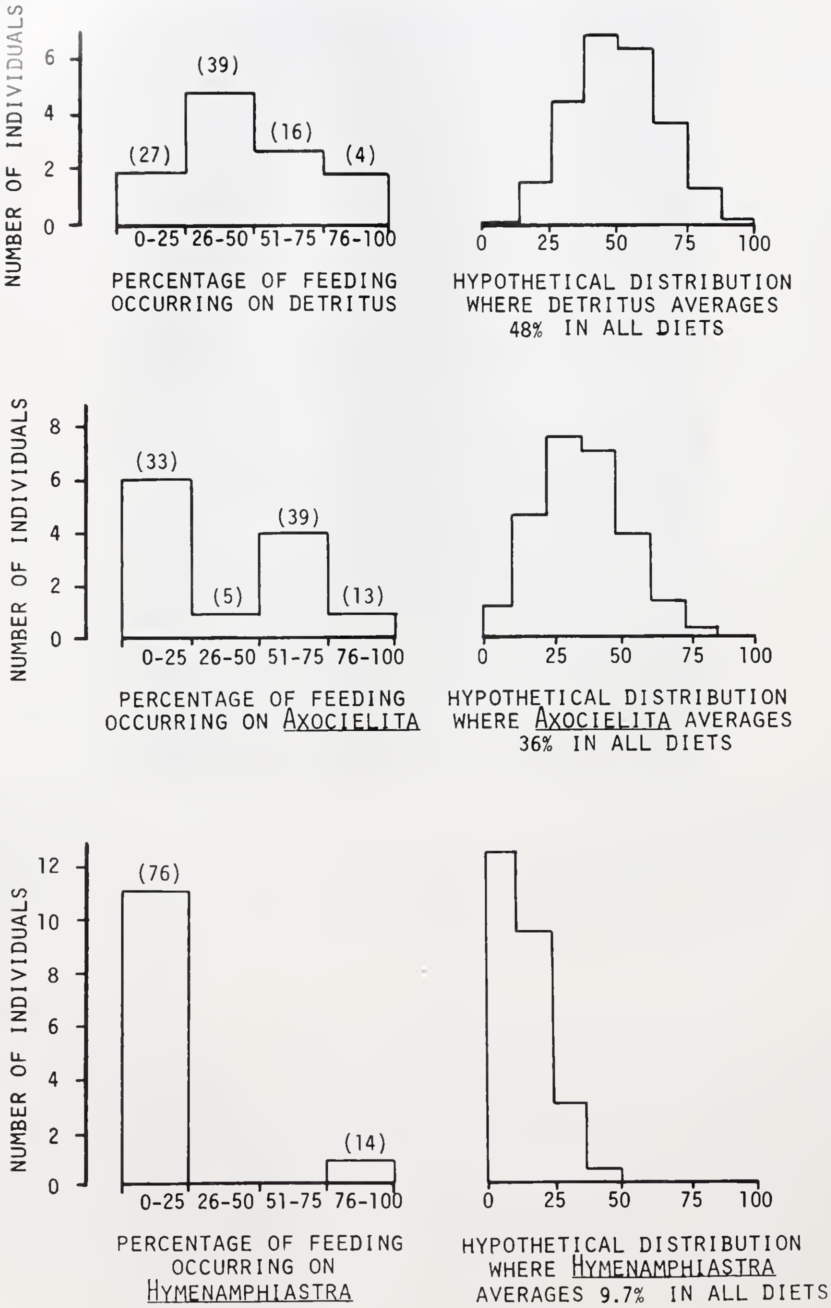

FIGURE 2. Frequency distributions of the numbers of individuals observed to be stationary (and probably feeding) on each food at various frequencies. Parentheses enclose numbers of observations. Kolmogorov-Smirnov test for goodness of fit between each observed and hypothetical distribution ($N = 90$): detritus $D = 0.105$, $P > 0.20$. *Axocielita* $D = 0.276$, $P < 0.05$. *Hymenamphiastra* $D = 0.428$, $P < 0.01$. *Astylinifer* (eaten only once in this experiment) is not analyzed.

term with n and i is each constant given by Pascal's triangle. Each product in the overall sum represents merely the expected probability of sampling an individual on a particular food (n − i) times during n observations (Kitting, 1980). If some individuals were to yield very few feeding occurrences and increase the variance of the actual distribution, one could sum hypothetical distributions, each for a single individual's n (the number of times it was observed stationary on food).

Comparing the hypothetical distribution to the actual numbers of individuals observed on each food (Fig. 2) shows that individuals tended to be stationary on detritus about 50% of the time. The detailed observations made over the 8-day period showed that detritus was eaten at intervals between feeding on a given sponge species (Table I). Furthermore, each individual was stationary on *Axocielita* either most of the time or very rarely. Only one individual appeared to feed on *Hymenamphiastra* in this experiment, and this nudibranch was never observed to eat any other sponge.

To confirm that different individuals tended to choose particular sponge species, even when alternative sponges were readily accessible, "transplant" experiments were conducted. Numerous nudibranchs were lifted carefully from *Axocielita, Astylinifer, Hymenamphiastra, Antho*, or detritus. If the mouth had been everted, the animal was placed (within 30 s) in a location where another individual had been feeding in previous observations. If individuals all had the same food preferences, those foods hypothetically most preferred would be eaten without regard to what the individual had been eating. However, individuals placed on another individual's feeding site or other sponges continued feeding only if the type of food remained the same; in 20 trials involving numerous individuals moved to another source of the same food, only six showed no mouth eversion (three to five trials for each of five foods). In contrast, individuals transferred to a different type of food rejected the other food in all 49 such trails (one trial for *Astylinifer* to *Antho*, one for *Antho* to *Astylinifer*, and two to five trials for each of 18 other combinations).

In a related test, two crawling (not feeding) individuals were placed successively on each of the same five foods, and allowed 5 min on each. One animal everted its mouth only when placed on *Hymenamphiastra*, and did this repeatedly, as though it had been seeking that food. The other crawling animal rejected each of the foods repeatedly. Although individuals differed somewhat in size, shade of yellow, and density of black markings, such differences showed no consistent relationship to the food preferred.

After the initial 8 days of routine sampling, most identified individuals could no longer be located. However, two nudibranchs were observed eating only *Hymenamphiastra* and detritus intermittently over 2 months. During the same 2 months, 2 of 10 individuals on specific rocks in sand were observed eating the sponge *Antho*, yet none of the nudibranchs on those rocks had eaten *Antho* during the earlier 8 days of the observations (see Animal R; Table I and Fig. 1).

In the laboratory tests, all individuals began eating *Astylinifer* within 1 week, even when other food sponges were present. However, no individuals were ever observed eating *Tethya aurantia* var. *californiana, Stelletta clarella*, or *Polymastia pachymastia*, although these species were among the most common sponges in the study areas. In the field, food species occurred in the following order of decreasing abundance: *Hymenamphiastra* > *Axocielita* > *Antho* > *Astylinifer* > *Myxilla*. The number of individuals observed eating each species during two months yielded the order: *Axocielita* > *Antho* > *Astylinifer* > *Hymenamphiastra* > *Myxilla*. Spearman's rank correlation coefficient showed no significant overall difference here

TABLE II

Bioassays of Anisodoris nobilis *digestive gland extracts. Ultrafiltrates were added to spontaneously beating guinea pig atria* in vitro. *Corresponding chemical analyses of histamine (after Shore, 1971) are noted where available. Decreases in heart action are in boldface type. Hist. = histamine.*

Description (and number of individuals)		Mean (and range) of mg extract in 8-ml bath	Heart action as mean (or range) of % initial action:	
			Amplitude	Rate
Fresh, unstarved *Anisodoris* feeding on:				
Axocielita	(3)	55 (33–66)	(133–180)	(100–130)
Astylinifer	(2)	90 (80–100) (with 0.4 μg hist./g)	(112–120)	(100–112)
Hymenamphistra	(1)	100 (with 0.7 μg hist./g)	151	148
Antho	(2)	67 (33–100) (with 0.5 μg hist./g)	(100–155)	(100–147)
Antho	(1)	133 (with 0.7 μg hist./g)	**54**	**70**
Myxilla	(1)	66	150	150
Detritus	(3)	93 (80–100)	**(55–80)**	(**50**–100)
Anisodoris starved 4 days (mean of 1–2 individuals from each of five sponge species)		65 (30–100)	135 (122–148)	114 (100–140)
Anisodoris starved 10–20 days (mean of 2–3 individuals from each of three sponge species)		67 (33–130)	**84** (**60**–115)	**81** (**61**–100)
Histamine standards		(0.050 μg hist./dose) (0.300 μg hist./dose)	123 169	irregular irregular

($r_s = 0.60$; 3 df; $P \approx 0.5$), since the nudibranch population ate these particular sponges in roughly their order of abundance, with the exception of the rarely eaten, common *Hymenamphiastra.*

Pharmacological properties of individual nudibranchs and foods

In pharmacological assays, the extract ultrafiltrates ranged from strongly excitatory to strongly depressant in their effects on contraction rate and amplitude of guinea pig heart. Table II compares results of assays using extracts of digestive glands of *Anisodoris* individuals. Differences among nudibranchs did not correspond to the sponge species being eaten at the time of collection. Extracts of individuals eating each sponge species caused some increase in rate and amplitude of the guinea pig heart in the assay, except for one seemingly normal individual eating *Antho.* This nudibranch and all three collected as they ate detritus produced a decreased rate and amplitude in the heart assay.

When *Anisodoris* specimens freshly collected as they ate sponges were starved for 2 days (half the period over which *Anisodoris* continued to defecate), ultrafiltrates of their digestive glands still yielded excitatory effects in the guinea pig heart assay. However, when such individuals had been starved 10–20 days, their digestive gland ultrafiltrates produced decreased heart rate and amplitude in the assay (Table II). As expected, analogous tests with feces themselves showed an excitatory effect in the heart assay.

TABLE III

Sponge extract ultrafiltrates assayed with guinea pig atria. Histamine (hist.) analyses are noted where available. Mean increases in heart action greater than the 130% average overall effect are in boldface type.

	Sponge (and number of replicates)		Mean (and range) of mg extract in 8-ml bath	Heart action as mean percent (and range) of % initial action: Amplitude	Rate
Eaten species	*Axocielita*	(4)	154 (44–220) (with 0.9 μg hist./g)	127 (107–153)	120 (100–138)
	Astylinifer	(2)	64 (27–100) (with 0.2 μg hist./g)	**139** (132–145)	115 (110–119)
	Hymenamphiastra	(4)	107 (27–220)	121 (100–157)	114 (100–120)
	Antho	(4)	110 (66–165)	108 (100–120)	96 (85–110)
	Myxilla	(1)	133 (with 0.2 μg hist./g)	125	**150**
Avoided species	*Stelletta*	(6)	95 (25–200)	**171** (146–187)	**137** (123–155)
	"(outer)		(200)	(187)	(142)
	"(inner)		(140)	(177)	(123)
	Tethya	(2)	88 (50–125) (with 0.3 μg hist./g)	**149** (135–162)	127 (125–128)
	Polymastia	(3)	133 (50–250)	**156** (113–188)	— (irreg.–128)
	Mean of eaten species		116	124	119
	Mean of avoided species		104	**159**	**132**

Table III summarizes analogous assays using ultrafiltrates of extracts of various food sponges plus similar extracts of three sponge species that *Anisodoris* avoided. All sponge species showed an excitatory effect in the guinea pig heart assay, although the effect of the sponge *Antho*, an acceptable food for *Anisodoris*, was very slight. Replicate experiments on single sponge colonies were not consistent enough to enable detailed comparisons between separate sponge colonies, but no major differences in results were evident among colonies of given sponge species. Overall, the three sponge species avoided by *Anisodoris* yielded 30% higher amplitudes and 10% higher rates in the heart assay, relative to the results from similar doses of readily eaten sponge species (Table III). Distinct outer and inner tissues of the avoided sponge *Stelletta* both caused roughly the same increase in the assay. Analogous tests with detrital food available to the nudibranchs produced no effect in the assay.

Histamine, which increases contraction rate and amplitude in the mammalian heart assay, was detected in extracts from sponges and *Anisodoris* digestive glands (Tables II and III). Histamine was present in eaten and avoided sponge species. Generally, enough histamine was present to account for excitatory effects of ultrafiltrates of both fresh *Anisodoris* and sponges in the heart assay. However, the amount of histamine did not parallel the strength of excitatory effect in the heart assay; apparently, other compounds also affected assayed heart action.

A methyl purine riboside named doridosine (N^1-methylisoguanosine) extracted from *Anisodoris* digestive gland strongly decreases contraction rate and amplitude of guinea pig atria (Fuhrman *et al.*, 1980). To determine if the compound could have been obtained from the nudibranch's food, sponge species eaten by *Anisodoris* were extracted and analyzed by the same methods. However, bioassays and thin-layer chromatography of column eluates revealed no doridosine or adenosine in any sponges tested (Table III). Fuhrman (personal communication) calculates that the sponges, if they contain the compound, have less than 1.6–2.2 μg purine riboside (as adenosine) per gram wet weight. This would be < 1% of the concentration in the nudibranch digestive gland.

Thus, the digestive gland extracts from starved nudibranchs, and from nudibranchs recently eating detritus, decreased rate and amplitude of muscle contraction in mammalian heart bioassays; this is a characteristic effect of doridosine. However, extracts from sponges showed an excitatory effect, characteristic of histamine detected in sponges.

Effects of food extracts on nudibranchs

In the field, nudibranchs periodically leave their feeding sites on sponges. It seemed possible that sponge exudates released during nudibranch feeding might exert some gross influence on the nudibranchs and stimulate them to leave. To test this, unfractionated sponge extract was applied to the animals externally, roughly simulating natural exposure. Three sponges were tested: the readily eaten *Axocielita*, the seldom eaten *Hymenamphiastra*, and the avoided species *Tethya*. Extracts were applied externally to two specimens of *Anisodoris* and two of *Doriopsilla*. Thirteen trials were made with each sponge extract. Seawater alone was applied as a control. Nudibranch heart rates and behaviors were then monitored 5–7 times per trial.

In these experiments, heart rates before and after each application averaged 44 ± 3 (SD) beats per 30-s observation. Analogous tests on the dendronotacean nudibranch *Dendronotus albus*, which does not eat sponges, also showed no change in heart rate. However, overt behavior of all the nudibranchs was clearly affected in the experiments. The application of each sponge extract was followed by each dorid or *Dendronotus* specimen retracting its appendages and moving away. The application of seawater as a control yielded no response. Applications of *Anisodoris* digestive gland extract produced clear movement away from the extract, but still did affect nudibranch heart rate.

Two analogous tests with *Anisodoris* bathed in 10^{-5} M serotonin suggested that such low-molecular-weight amine compounds produce little internal change when applied externally to the heart and gill region; heart rates remained near 44 ± 3 (SD) beats per 30 s upon applying serotonin externally, unlike serotonin's marked effect on clam heart (see Greenberg *et al.*, 1973). In marked contrast, external application of serotonin to nudibranchs caused marked external changes, including prolonged retraction of appendages and copious secretion of mucus from the animal's dorsal surface.

To test for toxicities of available foods, extracts of the sponges *Axocielita* and *Tethya* and of detritus available as food in the nudibranch habitat were prepared as described earlier. These were diluted 1:10 in seawater and injected into the nudibranch's pericardium *in vivo*. Each sponge or detritus extract was injected into two *Anisodoris* and two *Doriopsilla* specimens. Control animals (four *Anisodoris* and six *Doriopsilla* specimens) received only seawater injections. After injections of food extract, these animals were maintained in a 1:100 dilution of extract in seawater; this helped compensate for injected extract diffusing out of the nudibranch, and simulated internal plus external exposure to exudates of wounded sponge (or macerated detritus).

All injections, of sponge, detritus, or seawater only, produced temporary overall body contractions. No obvious change in heart rate occurred after seawater injections. Rates before and after injection averaged with ±3 beats (SD) of the initial 44 beats per 30-s observation (repeated 4–5 times for each individual). Detritus extracts likewise produced no significant change. In both nudibranch species injected with sponge, the most frequently eaten sponge (*Axocielita*) as well as the most conspicuously avoided sponge (*Tethya*) initially halted nudibranch heart action, although the nudibranchs continued to move about. The heart of *Anisodoris* began beating again intermittently after roughly 1 h. *Tethya* halted *Doriopsilla* heartbeat for over 12 h, after which heartbeats were intermittent. In both nudibranch species, *Tethya* injections produced thick mucus and violent body contractions. Serotonin (at 10^{-5} M in seawater, similarly injected into five specimens of *Anisodoris* and 5 *Doriopsilla*) temporarily halted heart action or produced no clear change. Only *Tethya*-injected nudibranchs died (two *Anisodoris* and two *Doriopsilla* specimens). Other individuals injected with food or serotonin appeared healthy while they were maintained in seawater for over 1 week following the experiments.

In summary, none of the sponge extracts showed marked effects on *Anisodoris* or *Doriopsilla* heart rate *in vivo* when applied externally, but all repelled the nudibranchs. When injected into *Anisodoris*, extracts of eaten and uneaten sponge species led to halted, then irregular heart beating, while avoided *Tethya* eventually killed *Anisodoris* and *Doriopsilla*.

Discussion

Anisodoris nobilis was the most frequent predator on sponges observed in the present study. It fed on at least five sponge species representing two families in the order Poecilosclerida. This nudibranch conspicuously avoided more than three common sponge species representing two other orders. *A. nobilis* at different localities appears to eat other sponge species (see McDonald and Nybakken, 1978) not common in the present study locality. Foraging differences observed among individuals in the present study did not correspond to differences in physical appearance or sex (all animals observed were mature and hermaphroditic). However, observations beyond the 8-day experiment show that *Anisodoris* individuals sometimes change their food species. The nudibranchs' different shades of color may possibly result from long-term dietary differences.

A striking finding was that different *Anisodoris nobilis* individuals tended to choose different sponge species, even though the nudibranchs traveled among the same set of available sponges for over 1 week. Analogous differences in the foods selected by conspecific individuals sharing the same food resources have been noted. Gain (1891), discussing the diets of land snails, remarked "perhaps individualism may account for some of the variation." Documented examples among invertebrates include work on insects reared on different plant species (reviewed by Hanson, 1976) and studies on bee foraging (reviewed by von Frisch, 1961, and Heinrich, 1976). It appears that such foraging differences among adjacent conspecific individuals may account for differences in natural, diet-dependent carbon isotope ratios of individual grasshoppers (Fry *et al.*, 1978). For marine invertebrates maintained in the laboratory, Landenberger (1968) noted that one *Pisaster giganteus* sea star consistently selected snails rather than the usual mussels as food, and Paine (1963) observed that one cephalaspid opisthobranch *Navanax* (*Chelidonura, Navarchus*) *inermis* consistently ate certain nudibranch species that all other individual *N. inermis* rejected. Studying carnivorous snails in the natural environment, Lani West (unpublished manuscript, Hopkins Marine Station) documented marked dietary differences between individual snails (*Nucella emerginata*) exposed to the same mussels, limpets, and barnacles.

Additional studies on more species are needed to determine how widespread such intraspecific resource partitioning is. It is tempting to account for the phenomenon itself by considering it analogous to the more familiar interspecific competition and resource partitioning (see Lawlor, 1980). In general, past interference competition (*e.g.* aggression) might have excluded neighboring individuals from a given animal's chosen resources, and favored evolution of such intraspecific resource partitioning.

However, no suggestion of interference or exploitative competition appears among these nudibranchs. Their intraspecific resource partitioning could be a simple consequence of several resources being available to the population, while an individual's specialization may be advantageous for locating, consuming, detoxifying, or digesting a distinctive food (Kitting, 1980).

Studies on diet mixing by individual limpets (Kitting, 1980) suggest that intraspecific resource partitioning may be uncommon among animals that avoid excessive amounts of given foods. The present results show that while individuals use some different food resources, they may avoid excessive, repulsive amounts of a single type of food by sharing detritus.

In the present study, the nudibranch's ability to relocate a particular feeding site after extended absence was partly responsible for observed differences in individual diets. It is unknown how these nudibranchs, or certain limpets that return to foraging sites (Kitting, 1978) and home sites (*e.g.* Breen, 1971) navigate back to previous sites.

Though the mammalian heart assay showed differences in chemical constituents among *Anisodoris* individuals, the differences did not correspond to the sponge species eaten. Instead, the unusual decreases in rate and amplitude of heart beat in the bioassay are associated with recent consumption of detritus, and with starvation in the laboratory. Apparently, varying amounts of histamine in the sponges (and in the digestive glands of fresh *Anisodoris*) mask the distinctive assayed effect of doridosine in the nudibranch's digestive gland. Analogous assays of *Anisodoris* extracts made from nudibranchs collected on different species of sponges at Santa Barbara, California, and at San Juan Island, Washington, suggest that this combination of doridosine and sponge histamine occurs in the nudibranchs over much of the geographic range of *Anisodoris* (Furhman, Fuhrman, and Kitting, unpublished data). Since no doridosine or another purine riboside, adenosine, was detected in the available sponges, the nudibranch appears to produce the compound or concentrate it over 100× from undetected trace amounts.

An Australian sponge, *Tedania*, is reported to contain doridosine in unspecified but extractable concentration (Gregson *et al.*, 1979). *Tedania* is in the same family as several sponges eaten in the present study: *Astylinifer, Hymenamphiastra*, and *Myxilla*. If individual nudibranchs or other animals each took up different compounds from different foods, each individual might benefit from the diverse collection of derived chemical defenses against higher-order consumers. Other, externally secreted compounds appear to be common defenses for nudibranchs (*e.g.* Thompson, 1976); fishes learn to avoid such colorful, distasteful objects as *Anisodoris nobilis* (see Kellogg, 1980). The defensive functions, if any, of internal compounds such as doridosine itself are not yet clear.

The bioassayed differences found among nudibranch digestive glands are associated with the animals' content of sponge histamine. After *Anisodoris* ceased feeding on sponge temporarily and ate detritus (apparently for less than a day), histamine was not detectable in the digestive gland. The same result appeared when *Anisodoris* had not eaten for 8 days. Presumably, other compounds from sponges also could be purged or destroyed relatively quickly during periodic feeding on detritus. The results of applying sponge extracts to *Anisodoris* suggested that nudibranchs are somewhat resistant to compounds in extracts of edible sponges, though they are not completely tolerant of sponge compounds.

In the mammalian heart assay, unpalatable sponges were somewhat more biologically active than edible sponges were, but one avoided sponge eventually killed *Anisodoris* and *Doriopsilla.* Moving away and eating detritus periodically could be advantageous to nudibranchs in preventing sustained, high external and internal levels of sponge constituents such as histamine. Furthermore, a nudibranch's rasping on sponge tissue seems by audible evidence infrequent, slow, and laborious (also see Forrest, 1953); periodic feeding on detritus might also be advantageous in preventing excessive exposure to sponge spicules.

Chemical findings suggested the use of *Anisodoris nobilis* for the present studies on intraspecific variation in chemistry and feeding. Pharmacological assays with a mammalian heart documented basic chemical differences among *Anisodoris* individuals recently eating sponges or detritus. Unusual pharmacological effects of doridosine in nudibranch digestive gland were the opposite of effects of major biologically potent compounds in the sponges, including histamine. Further chemical investigations may find even more potent compounds in unpalatable organisms such as sponges, and may find antidotes or antagonistic compounds in specialist predators (individuals or species) that eat these unpalatable organisms. However, a specialist predator's resistance to its chemically defended foods need not be complete, if additional foods such as detritus often purge noxious compounds from a predator's digestive system.

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LITERATURE CITED

BAKUS, G. J., AND G. GREEN. 1977. Ecology and toxicity of marine sponges. *Mar. Biol.,* **40:** 207–215.

BREEN, P. A. 1971. Homing behavior and population regulation in the limpet *Acmaea digitalis* (*Collisella digitalis*). *Veliger,* **14:** 177–183.

BURRESON, B. J., P. J. SCHEUER, J. FINER, AND J. CLARDY. 1975. 9-Isocyanopupukeanane, a marine invertebrate allomone with a new sesquiterpene skeleton. *J. Am. Chem. Soc.,* **97:** 4763–4764.

CHANG, C. W. J., A. J. WEINHEIMER, J. A. MATSON, AND P. N. KAUL. 1978. Adenosine as a causative asystolic factor from a marine sponge. Pp. 89–95 in P. N. Kaul and C. J. Sindermann, Eds., *Drugs and food from the sea.* University Oklahoma Press, Norman, Oklahoma.

CIERESZKO, L. S. 1970. Nitrogen compounds in Porifera and Coelenterata. Pp. 57–66 in J. W. Campbell, Ed., *Comparative biochemistry of nitrogen metabolism.* Academic Press, New York.

Faulkner, D. J., and C. Ireland. 1977. The chemistry of some opisthobranch molluscs. Pp. 23–34 in D. J. Faulkner and W. H. Fenical, Eds., *Marine natural products chemistry*. Plenum Press, New York.

Forrest, J. E. 1953. On the feeding habits and the morphology and mode of functioning of the alimentary canal in some littoral dorid nudibranch mollusca. *Proc. Linn. Soc. Lond.* **64:** 225–235.

von Frisch, K., 1961. *The dancing bees.* Harcourt, Brace, and Co., New York. 182 pp.

Fry, B., A. Joern, and P. L. Parker. 1978. Grasshopper food analysis: Use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. *Ecology* **59:** 498–506.

Fuhrman, F. A., G. J. Fuhrman, and K. DeRiemer. 1979. Toxicity and pharmacology of extracts from dorid nudibranchs. *Biol. Bull.* **156:** 289–299.

Fuhrman, F. A., G. J. Fuhrman, Y. H. Kim, L. A. Pavelka, and H. S. Mosher. 1980. Doridosine: A new hypotensive N-methylpurine riboside from the nudibranch *Anisodoris nobilis. Science* **207:** 193–195.

Gain, W. A. 1891. Notes on the food of some of the British molluscs. *J. Conch. Lond.* **6:** 349–360.

Greenberg, M. J., R. A. Agarwal, L. A. Wilkens, and P. J. B. Ligon. 1973. Chemical regulation of rhythmical activity in molluscan muscle. Pp. 123–142 in J. Salanki, Ed., *Neurobiology of the invertebrates.* Tihany Akademiai Kiado, Hungary.

Gregson, R. P., J. Quinn, and A. F. Cook. 1979. 1-Methylquanosine isolated from Australian sponge. (German Patent) *Chem. Abstr.* **91:** 39792.

Hanson, F. E. 1976. Comparative studies on induction of food preferences in lepidopterous larvae. Pp. 71–78 in T. Jermy, Ed., *The host plant in relation to insect behavior and reproduction.* Plenum Publishing, New York.

Harris, L. G. 1973. Nudibranch associations. Pp. 123–142 in T. C. Chang, Ed., *Current topics in comparative pathobiology*, Vol. 2. Academic Press, New York.

Heinrich, B. 1976. The foraging specializations of individual bumblebees. *Ecol. Monogr.* **46:** 105–128.

Kellogg, D. L., 1980. *Prey defenses and predator learning in the Nudibranchia.* M. A. Thesis, University of Texas at Austin. 98 pp.

Kitting, C. L. 1978. *Foraging of individuals within the limpet species* Acmaea (Notoacmea) scutum *at Monterey Bay, California, and the consequences on their mid-intertidal algal foods.* Ph.D. Thesis, Stanford University. 193 pp.

Kitting, C. L. 1979. The use of feeding noises to determine algal foods being consumed by individual intertidal molluscs. *Oecologia* **40:** 1–17.

Kitting, C. L. 1980. Herbivore-plant interactions of individual limpets maintaining a mixed diet of intertidal marine algae. *Ecol. Monogr.* **50:** 527–550.

Landenberger, D. E. 1968. Studies on selective feeding in the Pacific starfish *Pisaster* in southern California. *Ecology* **49:** 1062–1075.

Lawlor, L. R. 1980. Overlap, similarity, and competition coefficients. *Ecology* **61:** 245–251.

MacDonald, G. R., and J. W. Nybakken. 1978. Additional notes on the food of some California nudibranchs with a summary of known food habits of California species. *Veliger* **21:** 110–119.

MacFarland, F. M. 1905. Preliminary account of Dorididae of Monterey Bay, California. *Proc. Biol. Soc. Wash.* **18:** 38–39.

Minale, L. 1978. Terpenoids from marine sponges. Pp. 175–240 in P. J. Scheuer, Ed., *Marine natural products*, Vol. 1. Academic Press, New York.

Paine, R. T. 1963. Food recognition and predation on opisthobranchs by *Navanax inermis. Veliger* **6:** 1–8.

Perry, W. L. M. 1968. *Pharmacological experiments on isolated preparations.* Livingstone, Ltd., Edinburgh, Scotland. 131 pp.

Roughgarden, J. 1972. Evolution of niche width. *Am. Nat.* **106:** 683–718.

Shore, P. A. 1971. Determination of histamine. Pp. 89–98 in D. Glick, Ed., *Methods in biochemical analysis, suppl.* Interscience, New York.

Sokal, R. R., and F. J. Rohlf. 1969. *Biometry.* Freeman and Co., San Francisco. 776 pp.

Thompson, T. E. 1976. *Biology of Opisthobranch Molluscs.* Ray Society, London. 207 pp.

Reference: *Biol. Bull.* **161:** 141–151. (August, 1981)

DEVELOPMENT OF A "PRIMITIVE" SEA URCHIN (*EUCIDARIS TRIBULOIDES*): IRREGULARITIES IN THE HYALINE LAYER, MICROMERES, AND PRIMARY MESENCHYME[1]

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ABSTRACT

The normal embryonic development of the cidaroid sea urchin *Eucidaris tribuloides* from fertilization to the late two-armed larval stage is described with the aid of light and electron microscopy. Unusual developmental features include a virtual absence of a hyaline layer, irregularities in the number and size of micromeres, and a total lack of early mesenchyme (the so-called primary mesenchyme). These and other features are unlike developmental characters in the euechinoids ("modern" sea urchins). They are discussed with reference to the phylogeny of sea urchins. *Eucidaris tribuloides* may exhibit "primitive" developmental patterns representative of the cidaroid ancestor which was common to all living sea urchins.

INTRODUCTION

Nearly all extant sea urchins are members of the Subclass Euechinoidea, whose fossil record extends about 175 million years into the past. Although their precise phylogenetic origin is somewhat uncertain (Nichols, 1962; Durham, 1966), members of this subclass probably diversified rapidly soon after the Permo-Triassic extinction about 225 million years ago (Fig. 1). The ancestor of the euechinoids was a cidaroid sea urchin (Order Cidaroida), whose relatively undiversified survivors compose the Subclass Perischoechinoidea. The ancestral genus may well have been *Miocidaris*, the only echinoid definitely known to have survived the extinction. Today cidaroids form a relatively inconspicuous part of the marine fauna, usually occupying deep-water tropical habitats.

Since living cidaroids closely resemble the cidaroid ancestors of modern euechinoids, some of their characteristics, including any distinguishing features of their embryonic development, may be regarded as "primitive." Hence, study of cidaroid development may have phylogenetic relevance. In the well-studied euechinoids, development typically proceeds through a regular and characteristic 16-cell stage marked by a tier of 8 mesomeres in the animal hemisphere, a tier of 4 large macromeres, and a vegetal tier of 4 small micromeres (Hörstadius, 1973; Okazaki, 1975). Later, two populations of mesenchymal cells form. Micromeres produce primary mesenchyme, which emigrates from the wall of the late blastula into the blastocoel to give rise to the larvel skeleton. Secondary mesenchyme arises from the vegetal-most derivatives of the macromeres (*veg*$_2$); it emerges near the tip of the archenteron when the archenteron is about halfway invaginated, and forms the coelomic and other mesoderm.

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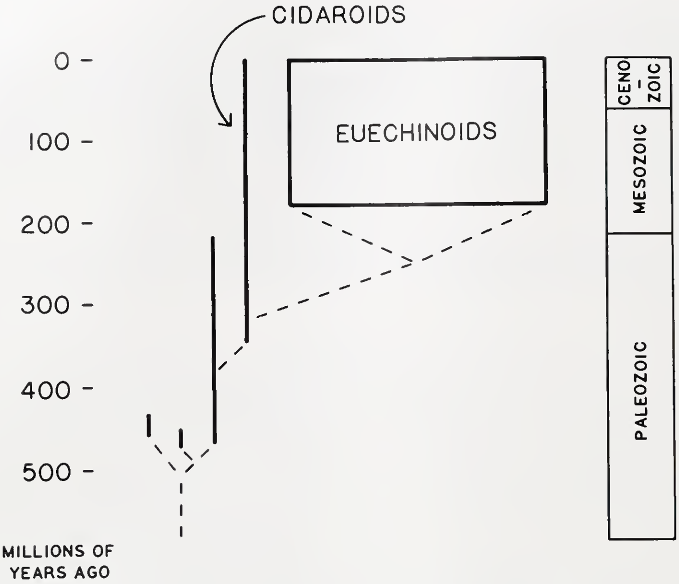

FIGURE 1. The phylogenetic relationship of cidaroid sea urchins to recent sea urchins (euechinoids), as simplified after Duncan (1966). The only known survivor of a massive extinction at the end of the Paleozoic was a genus of cidaroids; it may have been the common ancestor for both euechinoids and present-day cidaroids, or the ancestral cidaroid for euechinoids may have been earlier. Since the extinction, euechinoids have diversified extensively while cidaroids have not. Cidaroids which have survived to the present remain relatively "primitive."

Reproduction and development in cidaroids have received rather little attention (Holland, 1967; McPherson, 1968; Pearse, 1969). Tennent (1914, 1922) reported that embryos of *Eucidaris tribuloides* do not produce any primary mesenchyme, yet the larvae do form skeletons. The present article re-examines the development of this Caribbean cidaroid, using specimens from a shallow sub-tidal reef very near Tennent's original collection site. The study discusses certain unusual features of this urchin's development in light of the evolution of the more regular developmental pattern exhibited by other sea urchins.

MATERIALS AND METHODS

Eucidaris tribuloides adults (Fig. 2) were collected in Discovery Bay, Jamaica, during the second week of March 1980. They were typically found beneath or beside slabs of broken coral at depths of 2–3 m. Shedding of gametes was induced in the laboratory by intracoelomic injection of 0.5 *M* KCl. Of 80 adults collected, gametes were obtained from 4 males, 11 females, and 1 hermaphrodite. The remaining individuals contained no ripe gametes. Eggs washed twice in seawater were fertilized with dilute suspensions of sperm. Cultures were maintained in dishes in a sea-table or on a bench-top (26°C) or in an air-conditioned room (22°C). Seawater in the culture dishes was changed daily.

Eggs and embryos were photographed using Nomarski differential interference contrast microscopy, or bright-field microscopy between crossed polarizers for recording birefringence in the larval skeletons. To avoid compressing specimens during observations or photography, cover slips were supported on small daubs of

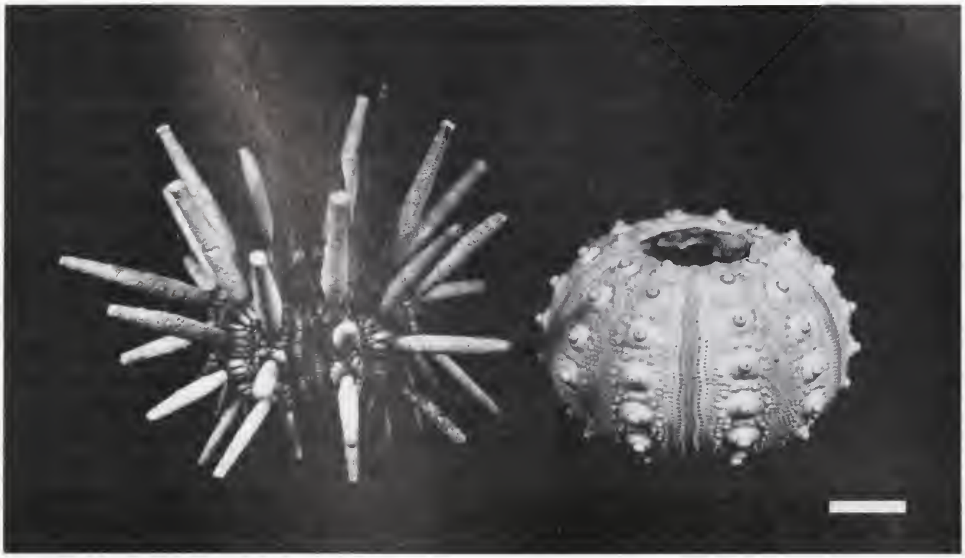

FIGURE 2. Life-size photograph of a small dried *Eucidaris tribuloides* (left) and the cleaned test of a large specimen (right). Note the heterologous spines which characterize this group. Scale bar = 1 cm.

modeling clay. Swimming specimens were immobilized with a small quantity of glutaraldehyde just before they were photographed.

Specimens were fixed in 2.5% glutaraldehyde–0.09 *M* sodium cacodylate–69% seawater pH 7.2 for 1 h, rinsed briefly in 0.2 *M* sodium cacodylate–50% seawater, and post-fixed in 1% osmium tetroxide–0.09 *M* sodium cacodylate–69% seawater for 1 h. They were then dehydrated and embedded in Epon for transmission electron microscopy, or critical point dried from CO_2 and coated with gold-palladium for scanning electron microscopy.

On occasion, fertilization envelopes were removed by pre-treating unfertilized eggs in 1 m*M* aminotriazole (Showman and Foerder, 1979) and then repeatedly passing them through a narrow-bore pipette about 30 min after fertilization. The eggs were then restored to seawater. This procedure successfully removed about 10% of the fertilization envelopes and caused the remainder to collapse. Development was normal after this treatment, even in the continuous presence of aminotriazole for 24 h.

RESULTS

The eggs of *Eucidaris tribuloides* are about 95 μm in diameter and are quite transparent. When the eggs were fertilized, a narrow perivitelline space 5–8 μm wide formed beneath the elevated fertilization envelope. A hyaline layer could scarcely be discerned at any time. Figure 3 shows stages of development at 26°C. First cleavage began at 50 min and subsequent cleavages occurred about 30 min apart. The 16-cell stage was visible at 2.5 h.

The embryos hatched at about 8 h, before the blastula could swim effectively. That natural dissolution of the fertilization envelope normally precedes active swimming was confirmed by rearing a culture directly on the microscope stage, thereby

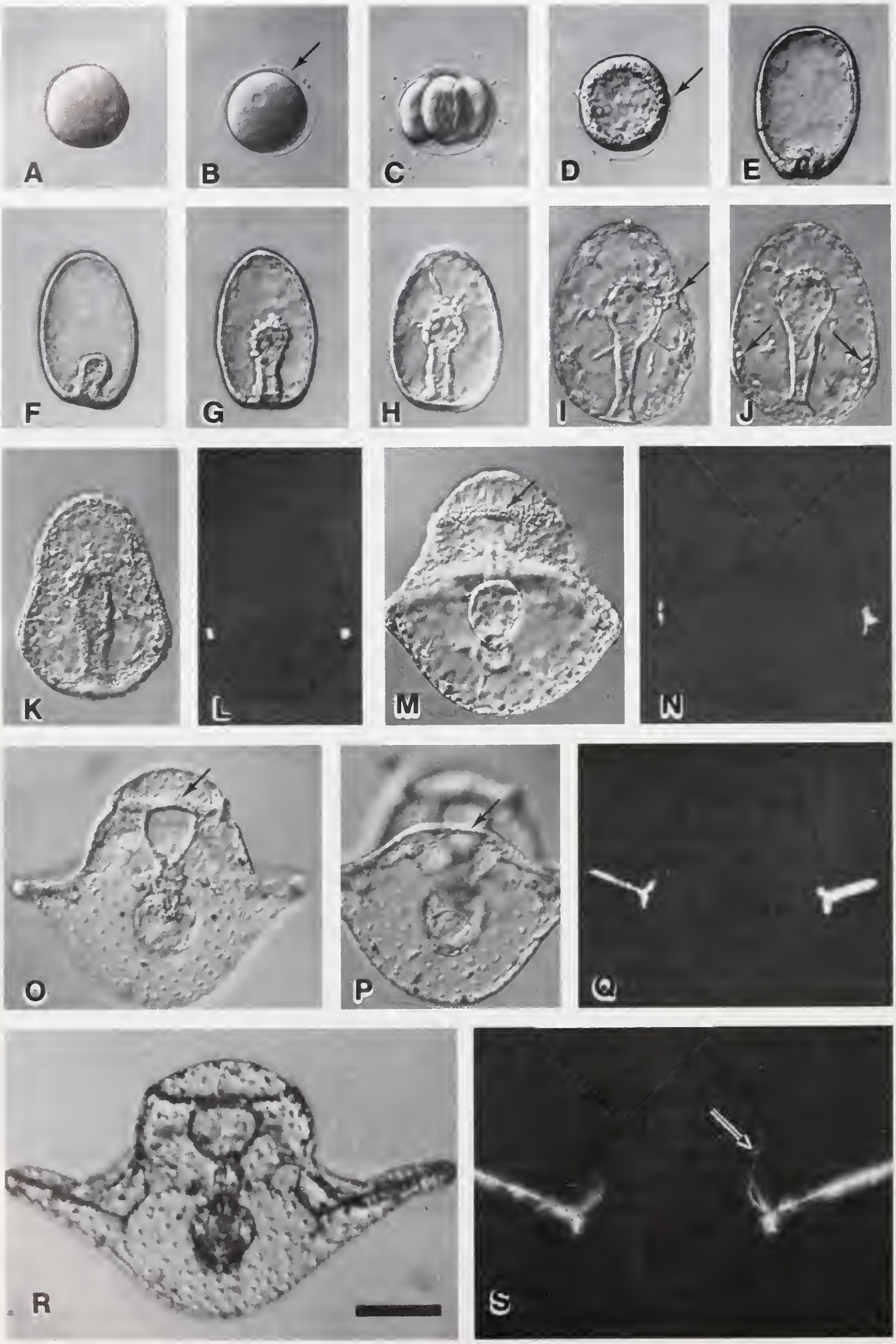

FIGURE 3. Stages of embryonic development of *Eucidaris tribuloides* cultured at 26°C. A: the unfertilized egg. B: the elevated fertilization envelope (arrow) 8 min after fertilization. C: the four-cell

ruling out the possibility that pipetting the embryos had artifically stripped off the envelopes. An apical tuft of cilia never formed after hatching. By 12 h swimming behavior was evident, but it remained sluggish throughout development.

As shown in Figure 3, the first sign of an invaginating archenteron was at about 14 h. The archenteron invaginated halfway before any sign of mesenchyme appeared. Even then, at 20 h, mesenchyme precursors were still attached to the archenteron tip. The blastocoel contained migratory mesenchyme cells by 22 h. By 40 h the archenteron contacted the stomodeum, and most of the mesenchymal cells were arrayed in the vicinity of this structure, except for two small clusters of cells situated bilaterally at the widest part of the embryo near the blastopore. By 44 h a birefringent spicule was detectable in each of these clusters. These spicules subsequently developed into the larval skeleton. In the next few days of development, the longest skeletal element was the post-oral rod, which in this species is fenestrated. Even after 7 days of culture, the slow-swimming larva was still two-armed and transverse rods did not join the bilateral skeletal elements. I never observed feeding, even when larvae were offered a suspension of mixed algae. A rudimentary ciliated band that appeared at about 70 h was complete by 75 h.

When eggs were cultured at 22°C, development was slowed so that the archenteron invagination began at 21 h and mesenchyme first appeared at 40 h. Despite this slow rate, the pattern of development was the same as at 26°C.

Figures 4–6 show selected aspects of development in greater detail. Figure 4 shows the virtual absence of the hyaline layer surrounding early embryos. Although microvilli about 1 μm long appeared over the entire surface a few minutes before first cleavage began, they were not obviously embedded in a detectable hyaline layer, even under completely normal conditions (Fig. 4A). When the fertilization envelope was mechanically removed, the shape of the early two-cell stage at the end of first cleavage (Fig. 4E) was considerably different from the shape when the constraining fertilization envelope was still intact (Fig. 4B). Without the fertilization envelope, the blastomeres were nearly spherical, further indicating the lack of a coherent hyaline layer. Subsequent development without a fertilization envelope resulted in disarrayed blastomeres (Fig. 4F, 5). Microvilli were readily visible by scanning electyron microscopy (Fig. 5B), even though no effort was made to remove the hyaline layer. This further attested to the virtual absence of a persistent hyaline layer.

Thin sections of eggs before and after fertilization (not illustrated) contained cortical granules that disappeared at fertilization. The contents of these cortical granules may be involved in forming the perivitelline space and in hardening the fertilization envelope, but either they contain little hyaline precursor or it readily solubilizes upon its release.

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stage at 1 h 48 min. D: the non-swimming blastula hatching out of the ruptured fertilization envelope (arrow) at 8 h. E: early stage of invagination at 14 h. F: the early gastrula at 18 h; note the absence of primary mesenchyme. G: the mid-gastrula at 20 h. H: the first signs of mesenchyme cells in the blastocoel at 21 h. I: lateral view from the right side when the archenteron tip contacts the stomodeum (arrow) at 39 h. J: the same embryo in frontal optical section showing two clusters of mesenchymal cells (arrows), which give rise to the primary spicules of the larval skeleton. K and L: the primary spicules appear as birefringent spots (L) in the early prism larva at 44 h. M and N: the advanced prism with developing ciliated band (arrow) near the mouth and enlarged tri-radiate spicules (N) at 69 h. O, P and Q: different focal levels of the young two-armed pluteus larva with a single complete ciliated band (arrows) and elongating post-oral rods (Q) at 75 h. R and S: the two-armed larva remains virtually unchanged between 100 and 180 h of culture, conceivably for lack of food; the antero-lateral rods of the skeleton (arrow) have formed in this 180 h specimen. A–S at the same magnification. Scale bar = 100 μm.

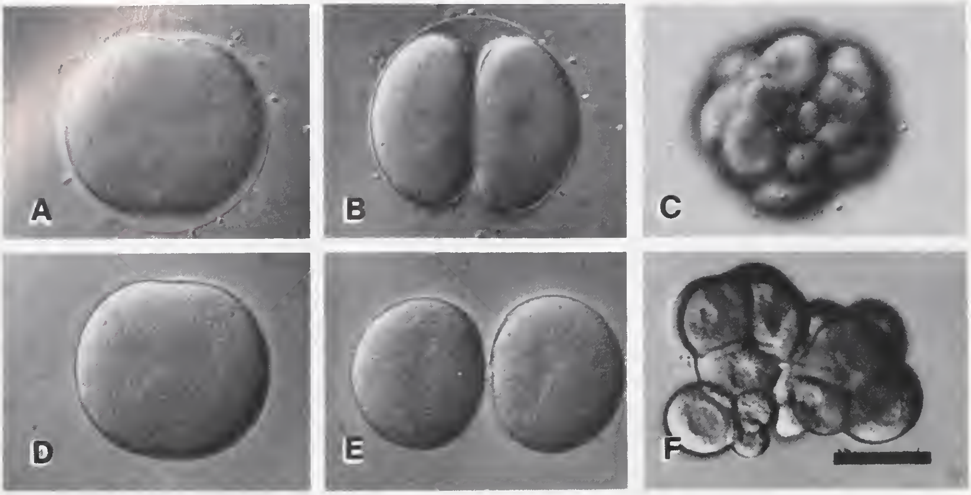

FIGURE 4. Details of early development when the fertilization envelope is intact (A–C) and after its removal (D–F). By light microscopy there is no evidence of a hyaline layer, even at the onset of first cleavage (A and D) when microvilli can be detected. When it is present, the fertilization envelope constrains the first two blastomeres into a roughly spherical shape (B), but without it the blastomeres separate widely (E). At the 16-cell stage there is an irregular number of micromeres (C and F) and the blastomeres are disarrayed; this is especially evident after the fertilization envelope has been removed and the embryo assumes a roughly rectangular configuration (F). A–F at the same magnification. Scale bar = 50 μm.

Figures 4 and 5 show the pattern of blastomeres at the 16-cell stage. Although micromeres formed, there were rarely 4 of them. Usually there were two or three micromeres of variable size and an equivalent number of macromeres, as judged by cell size. The remaining cells were all about the same size ("mesomeres"). Micromeres developed similarly, with or without fertilization envelopes, except that in the latter cases the embryos assumed a somewhat rectangular configuration (Fig. 4F, 5A), apparently because they were less confined. In scanning electron micrographs the surface of micromeres typically had few microvilli (Fig. 5B).

Closer inspection of blastulae and gastrulae (Fig. 6) confirmed that there was no evidence of mesenchyme cells in the blastocoel up to the mid-gastrula stage. When the archenteron was about one-third invaginated, some thin cytoplasmic projections reached into the blastocoel from cells near the tip (Fig. 6A). One-micron sections at a slightly later stage showed that the blastocoel was still devoid of cells, but filled with a loose meshwork of fibrils. Cells began to migrate into the blastocoel began at this stage (Fig. 6B).

DISCUSSION

This study confirms previous reports of the slow development of *Eucidaris tribuloides* (Tennent, 1914 and 1922; McPherson, 1968) and the unusual development of mesenchyme (Tennent, 1914 and 1922). Development in this species differs from that of more familiar euechinoid sea urchins in the following ways: (1) development is extremely slow; (2) very little hyaline material forms as the result of cortical granule release at fertilization; (3) blastomeres are separated widely after the first few cleavages, and are in disarray thereafter; (4) micromeres at the 16-cell stage are irregular in size and number, with corresponding effects on the appearance of macromeres; (5) hatching occurs before the blastula is very mobile; (6) there is no apical tuft of cilia; (7) no mesenchyme enters the blastocoel until the archenteron is halfway invaginated; (9) swimming at all stages is extremely slow; (10) the two halves of the larval skeleton remain unjoined at the midline for long periods.

Available evidence on the development of other cidaroids indicates that the pattern in *Eucidaris tribuloides* is representative of development in the Order Cidaroida generally. Prouho (1887) and Mortensen (1938) reported extremely slow development in other cidaroids. According to Mortensen (1938), the early blastomeres of *Prionocidaris baculosa* are widely separated, suggesting that the hyaline is reduced in this species, as it is in *Eucidaris tribuloides.* He also mentions that "in the 16–32 cells stage the cells were arranged in a rather unusual way, forming a two-layered plate" (Mortensen, 1938, p. 13). This recalls the rectangular appearance of embryos in Figures 4 and 5. He also reports that the number and distribution of the large macromeres and the small micromeres is variable, and that often no size difference can be detected (Mortensen, 1937).

The timing of mesenchyme migration into the blastocoel in cidaroids deserves additional clarification and comparative study. The absence of early mesenchyme in *Eucidaris tribuloides* may resemble the situation in *Prionocidaris baculosa,* according to a drawing of an early gastrula by Mortensen (1938). On the other hand, Prouho (1887) illustrates a similar stage of a Mediterranean cidaroid in which primary mesenchyme is specifically identified; the embryo also has an apical tuft, unlike *Eucidaris tribuloides.* The organism in the study was called *Dorocidaris papillata,* but has been renamed *Cidaris cidaris* (Mortensen, 1928).

The peculiar development of the mesenchyme in *Eucidaris tribuloides* suggests several possible interpretations. According to one view, it may indicate that this species entirely lacks any micromere-derived primary mesenchyme and that all migratory cells in the blastocoel are really secondary mesenchyme. This is not necessarily inconsistent with formation of a larval skeleton, since it has been shown (Hörstadius, 1973, p. 48, 50, and 61) that larvel spicules can still form in euechinoid embryos from which the micromeres have been experimentally removed. Apparently, cells originating from the *veg*$_2$ can organize spicules under certain circumstances.

This interpretation may be extended by postulating that *Eucidaris tribuloides* does not segregate the determinants of the micromeres and larval skeleton into the vegetal regions of the early embryo. Accordingly, the irregularities of the micromeres, the lack of primary mesenchyme, and the absence of an apical tuft could all be manifestations of an animal-vegetal axis that was imperfectly established at early stages (Schroeder, 1980).

According to an alternate interpretation, two separate populations of mesenchyme cells could happen to migrate simultaneously and subsequently sort out in the blastocoel. This would tend to obscure the distinction between primary and secondary mesenchyme, which is usually based on the times of migration rather than on cell lineage.

To determine which interpretation is correct may require experimental manipulations similar to those performed on euechinoids (Hörstadius, 1973). Such an approach would determine the role of micromere-derived cells in the pattern of

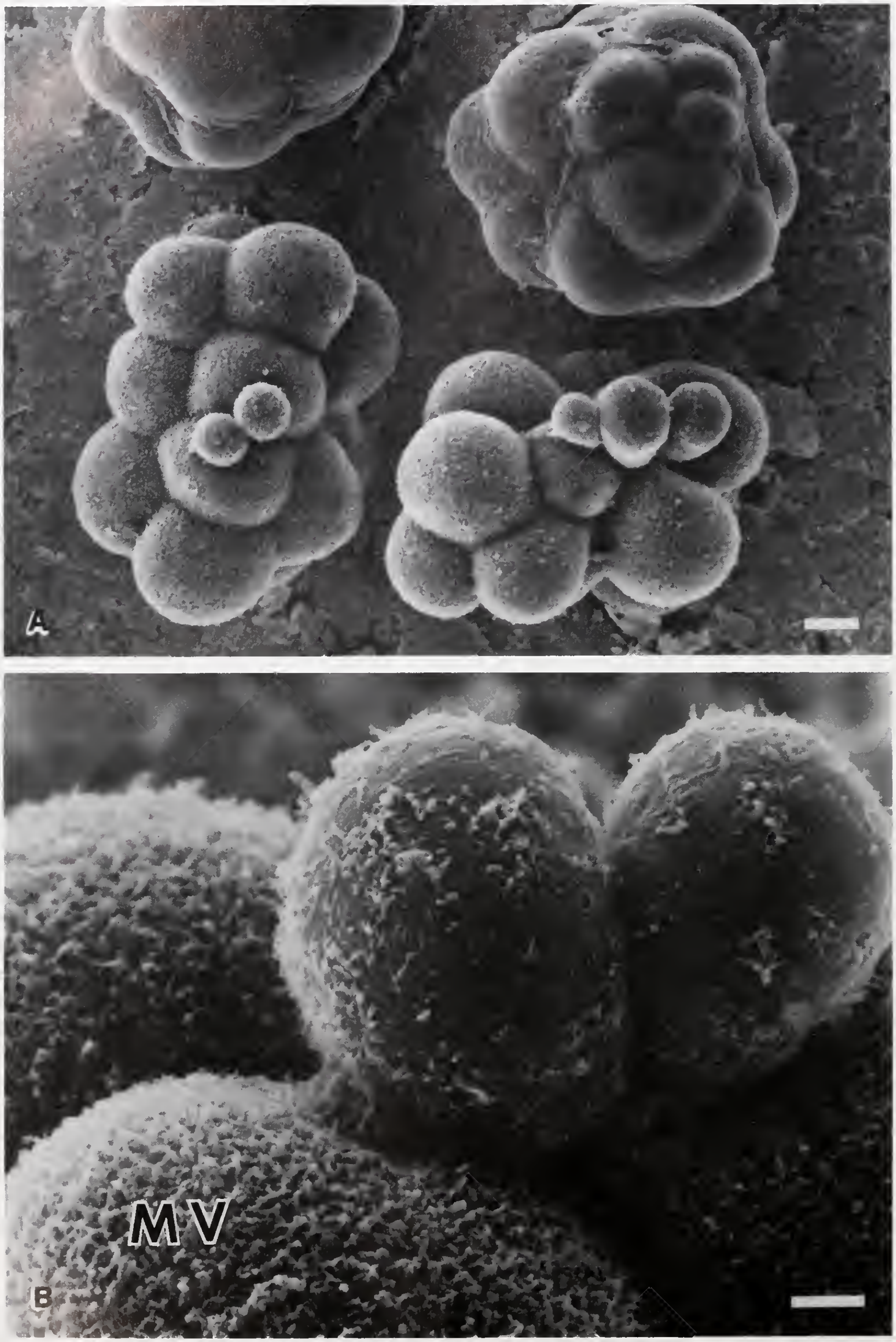

FIGURE 5. Scanning electron micrographs of embryos at the 16-cell stage, with fertilization envelopes removed (except for the two at the top of the figure). Embryos at this stage typically have 2 or

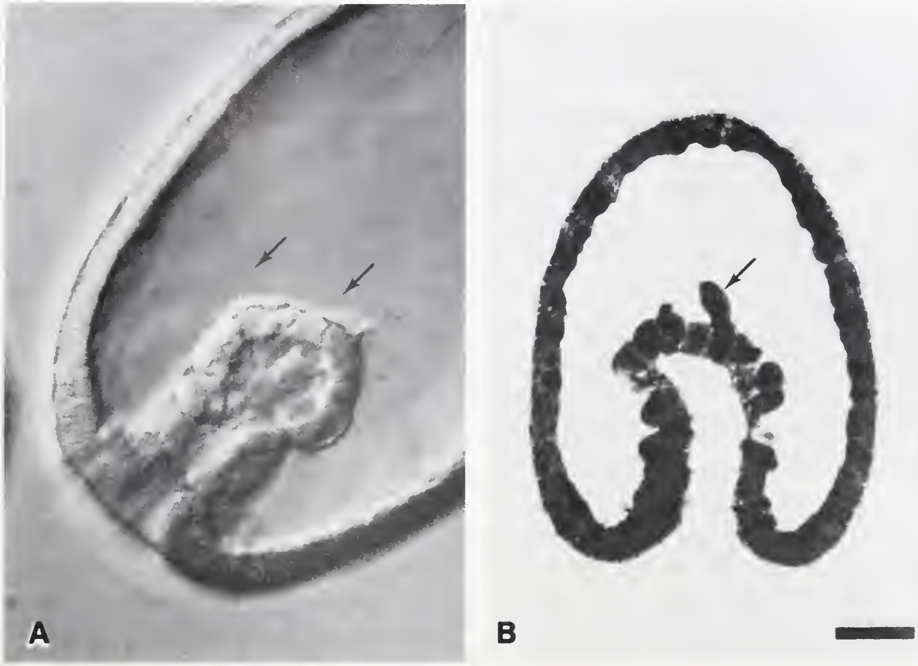

FIGURE 6. In *Eucidaris tribuloides*, mesenchyme cells do not enter the blastocoel until the middle stage of archenteron invagination. A Nomarski micrograph (A) of an early gastrula at 18 h reveals the lack of mesenchyme cells in the blastocoel; however, a few thin cytoplasmic processes (arrows) extend from the tip of the archenteron where mesenchyme cells will soon emerge. A 1 μm Epon section (B) of a mid-gastrula at 19 h (slightly shrunken during preparation) shows a precursor cell of the mesenchyme (arrow) shortly before it dissociates from the archenteron. A and B at the same magnification. Scale bar = 20 μm.

development. If skeleton-formation in cidaroids depends upon macromere-*veg*$_2$ cells rather than micromeres, this fact could help elucidate the phylogeny of the distinctive developmental pattern of euechinoids: It would indicate that the micromere-primary mesenchyme-larval skeleton system is a relatively recent evolutionary development.

Certain key developmental events differ greatly among the classes of the Phylum Echinodermata (Table I). But how these patterns relate to phylogeny is not clear. Only the euechinoids of Class Echinoidea have four micromeres at the 16-cell stage; these give rise to primary mesenchyme and then the larval skeleton. Ophiuroids do not form micromeres, but they still proliferate an early mesenchyme before archenteron invagination (Olsen, 1942). They also form a very prominent early larval skeleton very similar to the echinoid skeleton. Holothuroids (Oshima, 1921; Maruyama, 1980), crinoids (Seeliger, 1893), and asteroids (Dan-Sohkawa *et al.*, 1980) do not form micromeres or early mesenchyme, but some skeletal elements do appear in holothuroid embryos and crinoid larvae. Asteroid larvae never develop skeletons.

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3 micromeres, which vary in size, and an overall rectangular configuration (A). Microvilli are clearly visible on the larger blastomeres (B, MV), along with a minute amount of hyaline layer material. Scale bars = 10 μ (A) and 2 μm (B). Shrinkage has occurred during preparation.

Table I

Distribution of developmental features among the echinoderms. "Early mesenchyme" forms before or at the time of archenteron invagination. "Late mesenchyme" appears in the second half of archenteron invagination.

	Developmental features			
Taxon of Echinoderm	Micromeres at 16-cell stage	Early mesenchyme	Late mesenchyme	Larval skeleton
Class Holothuroidea	0	−	+	+
Class Echinoidea				
Subclass Euechinoidea	4	+	+	+
Subclass Perischoechinoidea (*Eucidaris tribuloides*)	1-4	−	+	+
Class Crinoidea	0	−	+	+
Class Asteroidea	0	−	+	−
Class Ophiuroidea	0	+	+	+

Several of the questions that arise in comparing the developmental pattern of *Eucidaris tribuloides* with development in other echinoderms (Table I) focus on the validity of homologies between mesenchyme populations and the cellular localization of skeleton-forming potential. A deeper analysis of this issue is necessary to understand the place of *Eucidaris tribuloides* in the Class Echinoidea, or the phylogenetic relationship between classes that might be discerned from embryonic development.

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I wish to thank Dr. Jeremy D. Woodley, Head of Discovery Bay Marine Laboratory, for his hospitality and assistance; Claudia E. Mills, who helped to collect specimens; and Dr. Robert L. Fernald, for reading the manuscript. This research was supported by U.S.P.H.S. research grant GM 19464.

LITERATURE CITED

Dan-Sohkawa, M., G. Tamura, and H. Mitsui. 1980. Mesenchyme cells in starfish development: effect of tunicamycin on their differentiation, migration and function. *Dev. Growth Differ.* **22:** 495–502.

Durham, J. W. 1966. Evolution among the echinoidea. *Biol. Rev.* **41:** 368–391.

Holland, N. D. 1967. Gametogenesis during the annual reproductive cycle in a cidaroid sea urchin (*Stylocidaris affinis*). *Biol. Bull.* **133:** 578–590.

Hörstadius, S. 1973. *Experimental embryology of Echinoderms.* Clarendon Press, Oxford. 190 pp.

Maruyama, Y. K. 1980. Artifical induction of oocyte maturation and development in the sea cucumbers *Holothuria leucospilota* and *H. pardalis. Biol. Bull.* **158:** 339–349.

McPherson, B. F. 1968. Contributions to the biology of the sea urchin *Eucidaris tribuloides* (Lamarck). *Bull. Mar. Sci.* **18:** 400–443.

Mortensen, T. 1928. *A Monograph of the Echinoidea. I. Cidaroidea.* Oxford University Press, London. 551 pp.

Mortensen, T. 1937. Contributions to the study of the development and larval forms of echinoderms. III. *Danske Videnskabernes Selskab Ser. 9,* **7(1):** 1–65.

Mortensen, T. 1938. Contributions to the study of the development and larval forms of echinoderms. IV. *Danske Videnskabernes Selskab Ser. 9,* **7(3):** 1–59.

Nichols, D. 1962. *Echinoderms.* Hutchinsen and Co., London. 200 pp.

OHSHIMA, H. 1921. On the development of *Cucumaria echinata*. *Quant. J. Microscop. Sci.* **65:** 173-246.

OKAZAKI, K. 1975. Normal development to metamorphosis. Pp. 177-232 in G. Czihak, Ed., *The sea urchin embryo*. Springer-Verlag, Berlin.

OLSEN, H. 1942. The development of the brittle-star *Ophiopholis aculeata* (O. F. Müller), with a short report on the outer hyaline layer. *Bergens Mus. Årbok* **6:** 1-107.

PEARSE, J. S. 1969. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. I. The echinoids *Prionocidaris baculosa* (Lamarck) and *Lovenia elongata* (Gray). *Bull. Mar. Sci.* **19:** 323-350.

PROUHO, H. 1887. Recherches sur le *Dorocidaris papillata* et quelques autres échinides de la Méditerranée. *Arch. Zool. Exp.* **5:** 214-380.

SCHROEDER, T. E. 1980. Expressions of the prefertilization polar axis in sea urchin eggs. *Dev. Biol.* **79:** 428-443.

SEELIGER, O. 1893. Studien zur Entwicklungsgeschichte der Crinoiden. *Zool. Jahrb.* (*Abt. Anat.*) **6:** 162-444.

SHOWMAN, R. M., AND C. A. FOERDER. 1979. Removal of the fertilization membrane of sea urchin embryos employing aminotriazole. *Exp. Cell Res.* **120:** 253-255.

TENNENT, D. H. 1914. The early influence of the spermatozoan upon the characters of echinoid larvae. *Carn. Inst. Wash. Publ.* **182:** 129-138.

TENNENT, D. H. 1922. Studies on the hybridization of echioids. *Carn. Inst. Wash. Publ.* **312:** 3-43.

Reference: *Biol. Bull.* **161**: 152–171. (August, 1981)

THE ALLOMETRY OF FEEDING, ENERGETICS, AND BODY SIZE IN THREE SEA ANEMONE SPECIES

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ABSTRACT

Three sea anemone species (*Anthopleura elegantissima, A. xanthogrammica, and Metridium senile*) were used to examine allometric and energetic properties of body size in passive suspension feeders. Photographs of expanded anemones in the field showed that projected feeding surface area (tentacle crown) as a function of body size increased at, or less than, the rate expected for a geometric solid (0.45–0.73 power or weight). Energetic cost, measured as weight loss, was found to relate differently to body size for each of the three species (0.77–1.08 power of weight).

Number of prey captured was closely related to the feeding surface area in all three species (0.36–0.7 power of weight). The exponent for prey biomass capture as a function of body weight was greater than that for energetic cost in *A. xanthogrammica*, (1.65 power of weight), but not for the other two species (0.33–0.54 power of weight). Prey size increased with predator size only in *A. xanthogrammica* (up to 10-cm anemone diameter), accounting for the higher increase in biomass capture. Numbers of prey captured by *A. xanthogrammica* continued to follow a surface-area function. Once the largest size classes of prey can be captured, further energy intake is probably directly related to feeding surface.

INTRODUCTION

As animals grow, their various appendages, organs, and physiological processes change at different rates (Thompson, 1917; Kleiber, 1932; Gould, 1966; Alexander, 1971; Schmidt-Nielsen, 1974). Few organisms grow as geometric solids, where all dimensions increase such that outward form remains the same (isometric growth). For example, feeding structures and absorptive surfaces can become convoluted or amplified, thus increasing their surface to volume ratio (Thompson, 1917; Gould, 1966). Energy balance, as intake minus cost, is determined in part by these geometric relationships.

Metabolic cost (as oxygen consumption) usually increases as a 0.60–1.0 power of weight, averaging 0.8 for marine invertebrates (Kleiber, 1932; Zeuthen, 1948a, 1953; Reichle, 1967; Vahl, 1972, 1973; McMahon, 1973; Jones, 1976; Bayne *et al.*, 1976; Newell *et al.* 1977). If energy intake increases as a lesser power of weight (*e.g.*, a surface area function) than does cost, the difference between the two (scope for growth, Warren and Davis, 1967; Vahl 1972, 1973; Kitchell *et al.*, 1977, 1978) rises to some maximum and then decreases (Sebens, 1977a, 1979, in press). Cessation of growth at this maximum point subsequently generates the greatest possible energy for reproduction. If energy intake increases as a greater power of weight than does energetic cost, there is no such obvious optimum individual size. However, allocation of all or most energy to reproduction may still cause individual size to asymptote.

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The relationship between energy intake, metabolic cost, and body size has been investigated in several actively filtering mollusks (Winter, 1969, 1973, 1978; McLusky, 1973; Vahl, 1972, 1973; Jørgenson, 1975; Bayne *et al.*, 1976; Newell *et al.*, 1977; Griffiths and King, 1979; Widdows, 1978; Thompson and Bayne, 1979) and ascidians (Randløv and Riisgard, 1979). Such relationships have not been investigated for any passive suspension feeder (*e.g.* corals, sea anemones, certain polychaetes, echinoderms, or gooseneck barnacles).

The present study quantifies feeding surface, prey intake, and energetic cost for three sea anemone species with different morphologies and habitat distributions: *Anthopleura elegantissima* (Brandt), *Anthopleura xanthogrammica* (Brandt), and *Metridium senile* (L.). The results are used to examine the control of body size as it relates to the species' habitat conditions. Some of the data on weight loss and prey capture in *A. xanthogrammica* have also been used to illustrate a more extensive general model of optimal body size for invertebrates with indeterminate growth (Sebens, in press) and are included here for comparison with the other two species.

MATERIALS AND METHODS

The anemone species

Anthopleura elegantissima is the most common intertidal sea anemone along the west coast of North America. It divides (longitudinal fission) to form clonal aggregations, sometimes with thousands of individuals (<1–6 cm diameter) (Hand, 1955a; Ford, 1964; Francis, 1973a, b, 1976, 1979; Sebens, 1977a, 1980; Jennison, 1979). Its prey include zooplankton, invertebrate larvae, and intertidal invertebrates (Sebens, 1977a). *Anthopleura xanthogrammica* is much larger (to at least 25 cm diameter intertidally), extends several meters into the subtidal in areas with extensive mussel beds, and never reproduces by fission (Hand, 1955a; Dayton, 1973; Sebens, 1977a, in press). It preys on mussels, sea urchins, barnacles, and other intertidal invertebrates (Dayton, 1973; Sebens, in press). Both *Anthopleura* species also harbor symbiotic algae (zooxanthellae and/or zoochlorellae) (Hand, 1955a).

Metridium senile is a primarily subtidal species forming large aggregations of individuals (to at least 18 cm basal diameter, 70 cm height) and sometimes reproducing by pedal laceration, especially in the low intertidal (Hand, 1955b; Hoffman, 1977; Purcell, 1977a; Shick and Hoffman, 1980). *M. senile's* large crown of numerous small tentacles is held away from the substratum in areas of appreciable current, so that water flows along the upper column, through the aboral side of the fluted oral disc, and across the tentacles (Koehl, 1976, 1977; Robbins and Shick, 1980) (Fig. 1). These anemones prey on zooplankton and invertebrate larvae (Purcell, 1977b; Sebens, 1977a).

Feeding surface area

Projected surface of the tentacle crown and total surface area of all tentacles were measured on a full size range of all three species. Anemones were photographed laterally and vertically in the field, while tentacles were fully expanded (*A. xanthogrammica*, *A. elegantissima* at Tatoosh Island, Washington, and *M. senile* at Harper, Washington). The projected surface of the tentacle crown described by the expanded tentacle tips was calculated as πr^2.

To calculate total surface area of all tentacles, tentacles of *A. xanthogrammica* and *A. elegantissima* were counted, and five were selected at random and measured

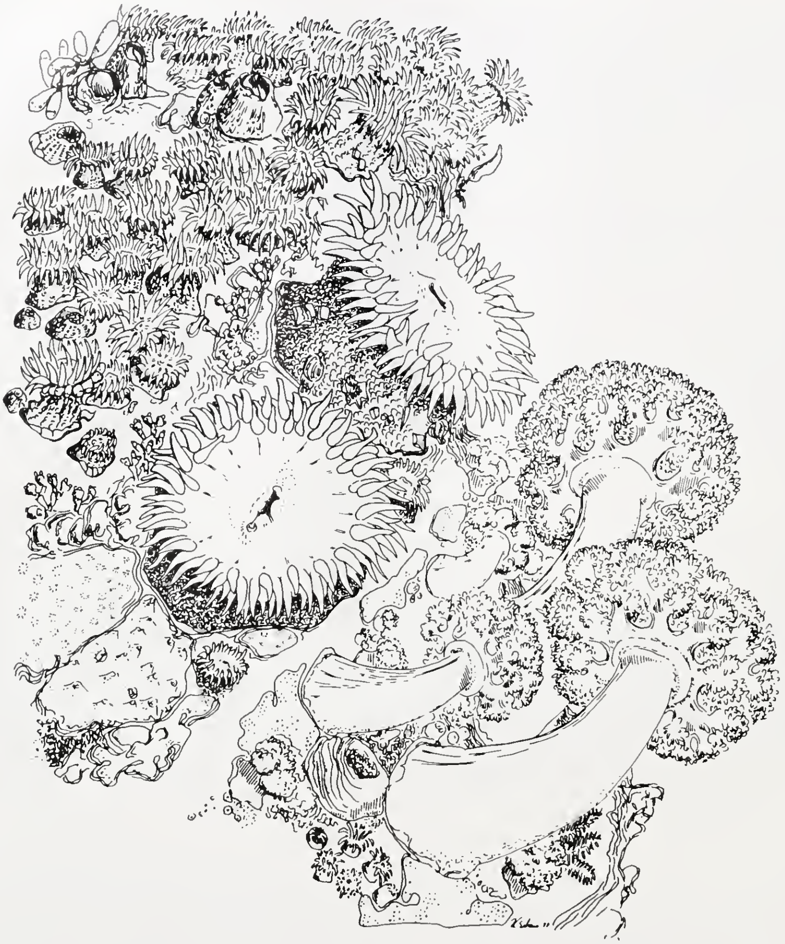

FIGURE. 1. Clonal aggregations of *Anthopleura elegantissima* (left) above *A. xanthogrammica* (middle) on a rock surface and *Metridium senile* (right) on a subtidal rock wall (positioned next to the others for comparison).

(tentacle length and width at mid-length). The surface of each tentacle was calculated as that of a cylinder, since much of the taper occurs near the tip in *M. senile* and *A. elegantissima.* A cone could have been used, but the difference between the two methods was less than 5%. Total tentacle surface was calculated as the number of tentacles multiplied by the mean surface area of the five single tentacles.

The tentacles of *M. senile* were too small and numerous to count from photographs. Individuals were measured in the field (basal diameter), collected, relaxed in 7.5% $MgCl_2$ (1:1 in sea water), and frozen. Depending on its size, the tentacle crown was subdivided into 4, 8, 16, or 64 parts, and three samples were chosen for tentacle counts. Tentacle number was calculated as the mean (±standard deviation) of the three samples (multiplied by the number of tentacle crown subdivisions) and tentacle surface as the number of tentacles multiplied by the surface area of a single tentacle. Correlations and principal axes were determined on double logarithmic transforms of the data to estimate the relationship as a power function of individual weight (Sokal and Rohlf, 1969).

Ash-free dry weight was calculated for 38 specimens of *A. elegantissima*, 16 of *A. xanthogrammica*, and 36 of *M. senile*, collected March 1976 on Tatoosh Island (*A. xanthogrammica* and *A. elegantissima*) and May 1976 at Harper (*M. senile*), of a full size range for that site and date. Attached-pedal-disc diameter was measured. The anemones were strung in order on monofilament line, and were brought alive to Seattle, where they were frozen at −20°C. They were later cleaned of adhering material, dried to a constant weight (less than 0.2% weight change per day) at 78°C ($T = 28$ days) to give total dry weight. They then were combusted at 500°C for 10 h to give ashed weight and ash-free dry weight. Principal axes were determined from double logarithmic transforms of pedal disc diameter versus ash-free dry weight.

Energetic cost

Anemone oxygen consumption depends on size, activity, state of expansion or contraction, illumination, temperature, and oxygen tension (Brafield and Chapman, 1965; Beattie, 1971; Sassaman and Mangum, 1973, 1974; Shick and Brown, 1977; Shick *et al.*, 1979; Robbins and Shick, 1980). Estimating metabolic cost as a function of body size using oxygen consumption requires numerous trials with anemones of known activity and history. Metabolic cost can also be estimated as weight loss per unit of original weight during starvation (Zeuthen, 1948b), thus averaging cost over a long time period, several activity levels, and periods of expansion or contraction. This method assumes that catabolic costs are approximately equal to, or less than, anabolic costs. Anemones spend much of their time without prey in the coelenteron (this study) and catabolic costs are likely to be applicable most of the time.

For the present study, 13 specimens of each species were marked with a pattern of small dye spots around their bases (Sebens, 1976) so that they could be identified in large aquaria. Three days later, they were weighed in sea water on a torsion balance (reduced weight method, W_r) (Holter and Zeuthen 1948, Zeuthen 1948b, Muscatine 1961), and placed in aquaria at 10°C (approximately 8 h light, 50–70 microeinsteins$\cdot m^{-2} \cdot sec^{-1}$) with aerated seawater filtered to remove particles greater than 20 μm. They were weighed again at 28 and 56 days (with sea water from the same batch used in the first weighing). Excess water was always removed from anemones before weighing by squeezing them gently to cause full contraction. Air bubbles were not allowed to enter the coelenteron or adhere to the surface of the anemones during weighing. Weight loss per individual per 28 days ($N = 26$ estimates) was compared to initial weight. Double logarithmic transforms of the data were used to plot regression lines and to estimate cost (tissue metabolized) as a power function of weight.

Prey capture

Capture rates and the relationship of such rates to individual size depend upon prey size, current or wave velocity, and a host of other factors that make laboratory measurements of limited usefulness. Therefore, prey capture success as a function of individual size was investigated in the field by sampling and by experimental prey release.

One thousand small mussels (3.0 ± 0.5 cm length) collected on Tatoosh Island

(March 1976) were marked with short file grooves across the valve closure, frozen, then distributed haphazardly back into the mussel bed at low tide a few meters above populations of *A. xanthogrammica*. Wave action during the next high tide washed these unattached mussels out of the bed, and many were captured by anemones. At the next low tide, anemones were sampled by probing their coelenterons, removing any contents, and checking mussels for file marks. One hundred anemones (3 cm or greater basal diameter) were examined at each of the two sites, and prey capture per individual anemone in each size class (every 2 cm diameter) was calculated.

Naturally captured prey of *A. xanthogrammica* were sampled by coelenteron probing over the period September 1974 to October 1977 at three population monitoring areas on Tatoosh Island (Sebens, 1977a). All items (>1 mm length) (usually wrapped in a mucous bolus surrounded by mesenterial filaments) were removed, measured, and identified. Several individuals were also examined for the presence of microscopic zooplankters (coelenteron sampled with a large syringe). Few were encountered and their total mass was insignificant compared to that of the macroinvertebrates. The results were plotted as dry weight of mussel tissue (from Fox and Coe, 1943) versus anemone weight for each anemone size class (mussels constituted more than 78% of the diet by weight).

Since *A. elegantissima* normally captures much smaller prey (zooplankton and small intertidal invertebrates), it was fed frozen adult brine shrimp (0.5 cm body length) which were of the appropriate size and were easily identified in coelenteron contents, as they were different from naturally occurring prey. Five pounds of frozen shrimp (purchased at a local aquarium supply store) were thawed in 5-gal buckets of sea water and dumped into the mouth of a surge channel at Shi Shi Beach, Washington (May 1976) in three pulses 5 min apart. Wave action dispersed the shrimp throughout the channel. After 30 min, anemones of a full size range were collected about 5 m landward from the mouth of the channel. The animals were placed in separate vials and fixed with 7% buffered formalin in sea water. Later, they were transferred to petri dishes, slit lengthwise to open the coelenteron, and all mesenterial filaments, coelenteron contents (food bolus), and many of the mesenteries were scraped into the dish. The contents of the petri dish and of the original vial (in case anemones egested prey) were systematically searched under a dissecting microscope. All brine shrimp, shrimp pieces, and naturally occurring prey were counted and measured with an ocular micrometer.

Scraping removed all coelenteron contents, and floating the fixed contents in a petri dish with about 2 mm water depth made prey items easy to identify and measure. This method is probably more accurately quantitative that the suction and filtration method for removing prey from corals (Porter, 1974) or zoanthids (Sebens, 1977b), because all prey and tissue to which prey might adhere were removed.

Prey items included planktonic larvae (barnacle cyprids), copepods, and various intertidal crustaceans, small bivalves, and barnacles. For comparative purposes, prey wet weight per individual anemone was calculated. Prey density was assumed to be close to that of sea water (0.00102 g/mm^3, Pickard, 1975) and prey volume was approximated as a cyprid-shaped geometrical solid (two cones with bases attached):

$$\text{Volume (mm}^3) = \pi L^3/192$$

$$\text{Wet weight (g)} = \text{volume (mm}^3)\cdot 0.00102\ (\text{g/mm}^3)$$

where L is prey item length in mm, and $\pi L^3/192$ is the volume of two cones with base diameter half their height. This comparative method of weight estimation considers both size and number of prey items in case prey size changes with predator size.

Metridium senile preys primarily on zooplankton. At Harper 10 lbs of frozen adult brine shrimp were thawed in two 5-gal buckets with lids. Divers using SCUBA gear took the buckets 2–3 m deep and released the shrimp in two pulses approximately 10 min apart 5 m up-current from an area (2–3 m depth) covered with *M. senile* specimens to 40 cm tall. After 20 min, 107 anemones were collected by two divers, basal diameters were measured, and the anemones were placed in individually marked vials or plastic bags. On shore, anemones were fixed in 7% buffered formalin in sea water (also injected into the coelenteron). Coelenteron contents were sampled and quantified as described for *A. elegantissima.*

Large solitary *A. elegantissima* anemones from Southern California (Arroyo Hondo) were examined for prey during May 1975. These anemones reach basal diameters to 16 cm and do not form clonal aggregations (Hand, 1955a; Sebens, 1977a; Francis, 1979).

Statistics

All statistical tests, regression analysis (with coefficient of determination; R^2), Pearson's product-moment correlation (with correlation coefficient, R) and principal axis determinations were calculated as described by Sokal and Rohlf (1969). All power functions were determined from double logarithmic plots of the data and 95% confidence intervals for the slope of the regression or principal axis were calculated. These are also the confidence limits for the exponent in the power function and they are given with each graph of the data.

RESULTS

Feeding surface area

Ash free dry weight to basal diameter correlations (principal axis) were as follows:

A. elegantissima $W_d = 0.0188\ D^{3.27}$ $R = 0.96$ $N = 38$

A. xanthogrammica $W_d = 0.0346\ D^{2.67}$ $R = 0.94$ $N = 16$

M. senile $W_d = 0.0073\ D^{2.78}$ $R = 0.88$ $N = 36$

where W_d is ash-free dry weight (g), D is diameter (cm), N is number of individuals, and R is the correlation coefficient.

As they became larger, *Anthopleura xanthogrammica* and *A. elegantissima* produced new tentacles (to 6 cycles) in a hexamerous arrangement. The maximum number was reached rather quickly (*A. xanthogrammmica,* 218 ± 42 (SD), N = 17; *A. elegantissima,* 119 ± 17 (SD), N = 18, Fig. 2). Above that size, tentacle length and width increased so that tentacle surface continued to increase without an increase in tentacle number. For *A. xanthogrammica,* tentacle surface area increased as the 0.87 power of individual weight (N = 19, R = 0.95) and for *A. elegantissima* as the 0.54 power (N = 17, R = 0.89) (Fig. 3). The difference appeared to depend on tentacle growth in length and width, which is proportionally greater in *A. xanthogrammica.* For *M. Senile,* however, tentacle number (N_t) increased as the 0.84 power of weight (N = 19, R = 0.99) (Fig. 2) as did tentacle

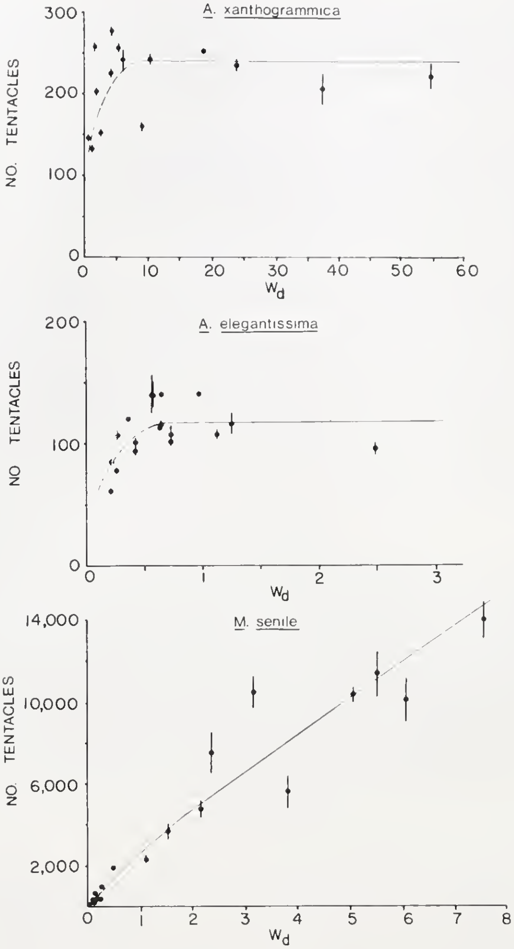

FIGURE 2. Number of tentacles as a function of individual ash-free dry weight (grams). Bars = ± one standard deviation (SD) for three measurements.

A. *A. xanthogrammica* (N_t = 218 tentacles ± 42 (SD), N = 15)

B. *A. elegantissima* (N_t = 119 tentacles ± 17 (SD), N = 19)

C. *M. senile* ($N_t = 2673\ W_d^{0.84}$, R = 0.99, N = 19, $P < 0.001$). The 95% confidence limits for the exponent of W_d are 0.77 and 0.91.

surface (Fig. 3). Tentacle size was similar for *M. senile* of a range of sizes (6.0 ± 1.0 mm tentacle length, 0.8 ± 0.1 mm width).

Projected oral surface of *A. xanthogrammica* increased as the 0.74 power of weight (N = 17, R = 0.98), of *A. elegantissima* as the 0.43 power of weight (N = 17, R = 0.93) and of *M. senile* as the 0.72 power of weight (N = 42, R = 0.93). Although two of the values are close to a geometric surface area (0.67) relationship, that for *A. elegantissima* falls well below it (Fig. 3). Slower increase of *A. elegantissima's* tentacle length probably accounts for this difference.

Energetic cost

Weight loss as a function of initial weight increased as a higher power of weight than did projected oral surface area in each species. Cost increased as the 1.08

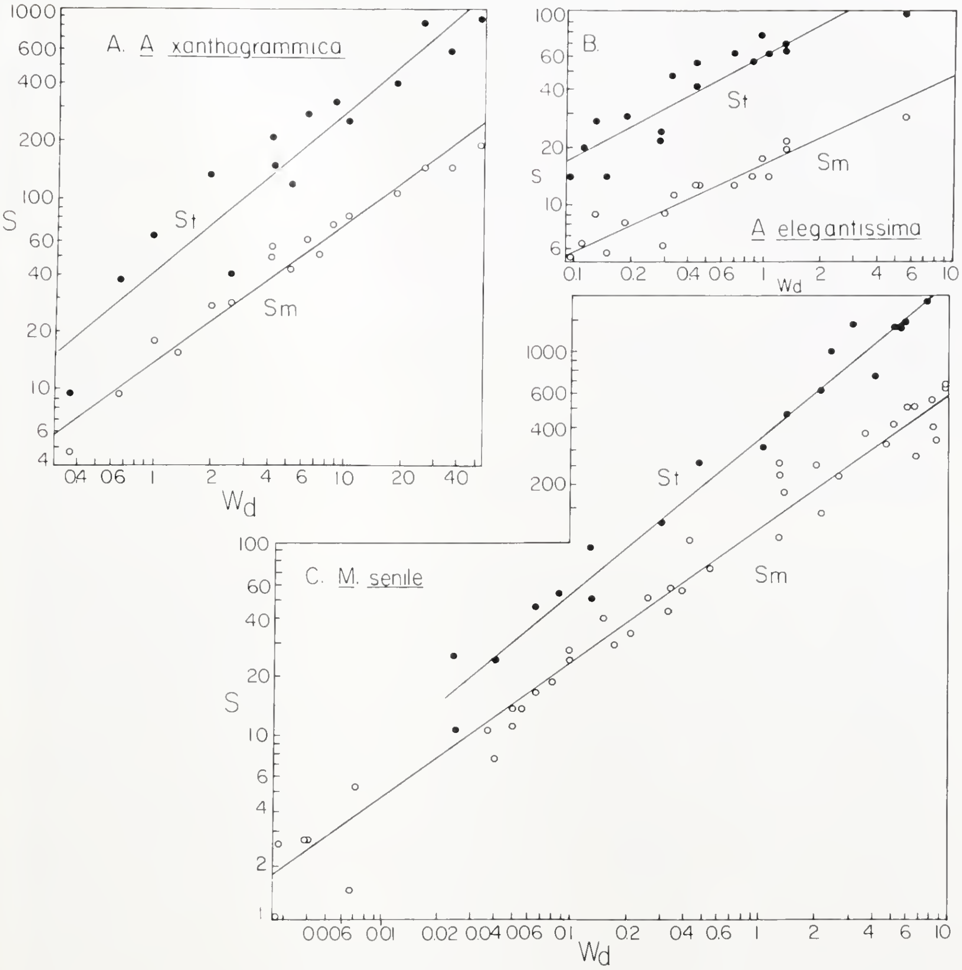

FIGURE 3. Projected oral surface of tentacle crown (S_m) and surface area of tentacles (S_t). Principal axis lines and correlation coefficients (R, all significant at $P < 0.001$) determined from double logarithmic transforms. Numbers in parentheses are 95% confidence limits for the exponent of W_d.

A. *A. xanthogrammica*, $S_m = 13.9\ W_d^{0.74}$, (0.67, 0.81), N = 17, R = 0.98

$S_t = 41\ W_d^{0.87}$, (0.73, 1.03), N = 15, R = 0.95.

B. *A. elegantissima*, $S_m = 15.6\ W_d^{0.43}$, (0.35, 0.51), N = 17, R = 0.93

$S_t = 58\ W_d^{0.54}$, (0.42, 0.68), N = 17, R = 0.89.

C. *M. senile*, $S_m = 119\ W_d^{0.72}$, (0.68, 0.76), N = 42, R = 0.98

$S_t = 353\ W_d^{0.84}$, (0.77, 0.91), N = 19, R = 0.99.

power of weight for *A. xanthogrammica* (N = 21, $R^2 = 0.96$), as the 0.77 power of weight for *A. elegantissima* (N = 19, $R^2 = 0.92$), and as the 0.80 power of weight for *M. senile* (N = 24, $R^2 = 0.90$) (Fig. 4). These results agree well with values reported for other invertebrates and for fish (Kleiber, 1932; Zeuthen, 1948a, 1953; Jones, 1976). The exponent was also very close to that reported for oxygen consumption in *A. elegantissima* (Shick *et al.*, 1979).

Prey capture

A. xanthogrammica and *A. elegantissima* captured experimentally released

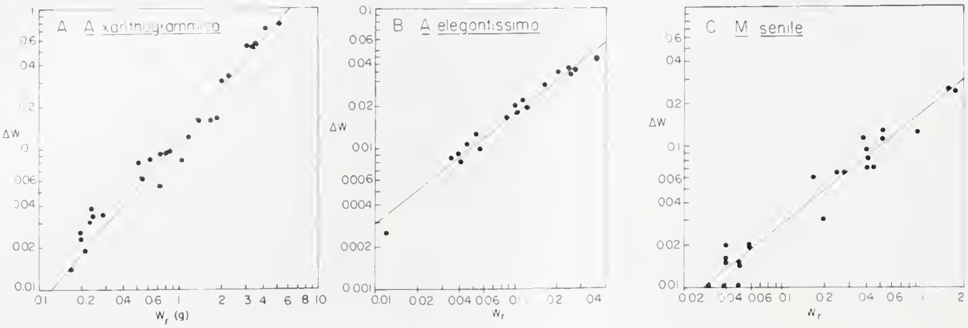

FIGURE 4. Weight loss at 10°C (change in reduced weight ΔW (g)) over 28 days, as a function of original weight (W_r(g)). Regression lines and coefficients of determination (R^2) were calculated from double logarithmic transforms. Numbers in parentheses are 95% confidence limits for the exponent of W_r.

A. *A. xanthogrammica*, $\Delta W = 0.123\ W_r^{1.08}$, (0.99, 1.17), N = 27, $R^2 = 0.96$
B. *A. elegantissima*, $\Delta W = 0.105\ W_r^{0.77}$, (0.69, 0.84), N = 19, $R^2 = 0.92$
C. *M. senile*, $\Delta W = 0.170\ W_r^{0.80}$, (0.72, 0.87), N = 24, $R^2 = 0.90$

prey in large quantities (171 items per 200 anemones, and 29 items per 112 anemones, respectively). *M. senile,* however, captured only 16 items per 107 anemones. Coelenteron-content analysis showed that the experimental prey items of *A. xanthogrammica* (Table I) and *A. elegantissima* (Table II) were well within the size range of naturally occurring prey but that the adult brine shrimp were much larger than natural *M. senile* prey (5 mm vs. 1.5 mm mean length) (Table III).

Prey capture by *A. xanthogrammica* was proportional to the 0.67 power of weight, by *A. elegantissima* to the 0.36 power of weight, and by *M. senile* to the 0.71 power of weight. To compare capture rates, I assumed that naturally occurring prey in the coelenteron contents represented the same feeding period for all anemone sizes. This seems likely because, given the digestive machinery of sea anemones (prey are wrapped in mesenterial filaments which secrete enzymes onto the prey and engulf particles of material into the digestive endodermal layer), large and small anemones probably digest the same prey item in equal time. Number of naturally occurring prey captured by *A. xanthogrammica* ($\text{prey} \cdot \text{individual}^{-1} \cdot \text{feeding period}^{-1}$) was proportional to the 0.73 power of weight (Fig. 5). For *A. elegantissima* it was proportional to the 0.18 power and for *M. senile* it was proportional to the 0.59 power. However, natural prey capture in this species increased with size over the first six size classes (as the 1.19 power of weight; $R^2 = 0.94$) and decreased significantly over the last five size classes (as the −1.65 power of weight, $R^2 = 0.96$). Large and small individuals were intermingled but probably not so crowded as to interfere with each other's prey capture. Collections were made during moderate current velocity (<10 cm/sec), and large individuals may do better in higher velocities. The large number of prey in coelenterons (538 prey in 107 anemones) indicated that this was a real phenomenon on that particular day. In addition, Bucklin and Hedgecock (in press) have shown that there is a third *Metridium* species on the Washington Coast. It may be that the smallest individuals in this study are of the clonal species (*M. senile*) while the majority belong to the large, typically subtidal, nonclonal species (as yet undescribed).

Natural prey in coelenteron contents, transformed to relative prey weight per individual (grams/individual) indicates the effect of changing prey size on intake rate. Natural prey weight was proportional to anemone weight to the 1.65 power

TABLE I

Anthopleura elegantissima *prey items (>50 μm length) from coelenteron samples, Shi Shi Beach, Washington, 1976 (112 anemones, 113 prey items).*

Prey items	No. items	Prey length (mm)	% of items
Crustaceans:			
Barnacle cyprids	2	0.6–0.8	1.8
Balanus glandula	4	2.0–4.0	3.5
B. cariosus	1	6.0	0.9
Barnacle molts	4	2.0–2.5	3.5
Amphipods	4	0.6–4.0	3.5
Isopods	7	1.0–3.0	6.3
Copepods	3	2.5–4.0	2.7
Decapod zoea	1	2.0	0.9
Pugettia sp.	2	4.0	1.8
Cancer sp.	1	5.0	0.9
Decapod fragments	6	2.0–9.0	5.3
Crustacean fragments	8	0.8–1.8	7.1
Molluscs:			
Littorina scutulata	3	4.0–6.0	2.6
L. sitkana	6	1.0–6.0	5.2
Mytilus edulis	13	2.0–11.0	11.6
Mytilus californianus	1	1.5	0.9
Adula californiensis	1	2.0	0.9
Other bivalves	3	1.8–3.0	2.4
Acmaea scutum	1	5.0	0.9
Acmaea pelta	1	2.5	0.9
Chitons	6	4.0–11.0	5.3
Hydroid colony fragments:	8	2.0–12.0	7.1
Bryozoan colony fragments:	9	2.0–10.0	7.9
Polychaetes:	5	3.0–14.0	4.4
Spirorbis sp.	3	2.5	2.7
Platyhelminthes:	1	3.0	0.9
Echninoid spine:			
Strongylocentrotus purpuratus	2	5.0–6.0	1.8
Foraminifera:	2	0.5–1.5	1.8
Eggs:	3	1.0	2.7
Insect fragments:	1	6.0	0.9
Mite:	1	2.0	0.9
Plant Material: (algae, *Zostera*, terrestrial plant fragments, probably undigested)	60	1.0–16.0	not included

for *A. xanthogrammica,* to the 0.33 power for *A. elegantissima,* and to the 0.54 power for *M. senile* (Fig. 6). Prey capture by *M. senile* by weight increased over the first seven size classes, then decreased. It was proportional to the 1.16 power for the first seven size classes and to the −2.56 power for the last four size classes.

TABLE II

Anthopleura xanthogrammica *prey items (>1 mm length) from coelenteron samples, Tatoosh Island, Washington, 1974–1975 (481 anemones, 177 prey).*

Prey items	No. items	Prey length (cm)	% of items
Mussels:			
(*Mytilus californianus*) with some *M. edulis*)	122	0.8–10.0	68.9
Barnacles:			
(*Balanus cariosus, B. glandula*)	24	0.8–3.0	13.6
Barnacle molts:	18	0.8–1.4	10.1
Decapods:			
Petrolisthes sp.	4	1.0–3.0	2.3
Gastropods:			
Acmaea spp.	2	1.4–2.0	1.1
Amphissa columbiana	1	1.0	0.6
Ceratostoma foliatum	2	4.4–5.5	1.1
Tegula funebralis	1	2.5	0.6
Colonial ascidians	2	4.0–6.0	1.1
Echinoids:			
Strongylocentrotus purpuratus	1	3.0	0.6

Prey sizes remained essentially the same for all sizes of *M. senile* (1.5 ± 0.3 mm SD, N = 538) and *A. elegantissima* (4.4 ± 3.2 mm SD, N = 17) but increased for *A. xanthogrammica* (R = 0.44, $P < 0.01$, N = 122). Comparing only the largest

TABLE III

Metridium senile *prey items (>50 um length) from coelenteron samples, Harper, Washington, 1976 (107 anemones, 538 prey).*

Prey items	No. items	Prey length (mm)	% of items
Crustaceans:			
Barnacle cyprids (*Balanus cariosus, B. glandula, Cthamalus dalli*)	380	1.0–1.8	75.2
Barnacle nauplii:	6	0.6–1.2	0.9
Copepods	71	0.6–1.2	11.1
Decapod zoea larvae	25	1.6–2.6	3.9
Eggs:	28	0.2–1.2	4.4
Bryozoan colony fragments:	8	2.0–3.0	1.3
Gastropod veligers:	6	1.4–1.8	0.9
Bivalve veligers:	8	1.0–1.4	1.3
Polychaetes:	3	3.0–6.0	0.5
Asteroid bipinnaria:	3	1.0–1.2	0.5

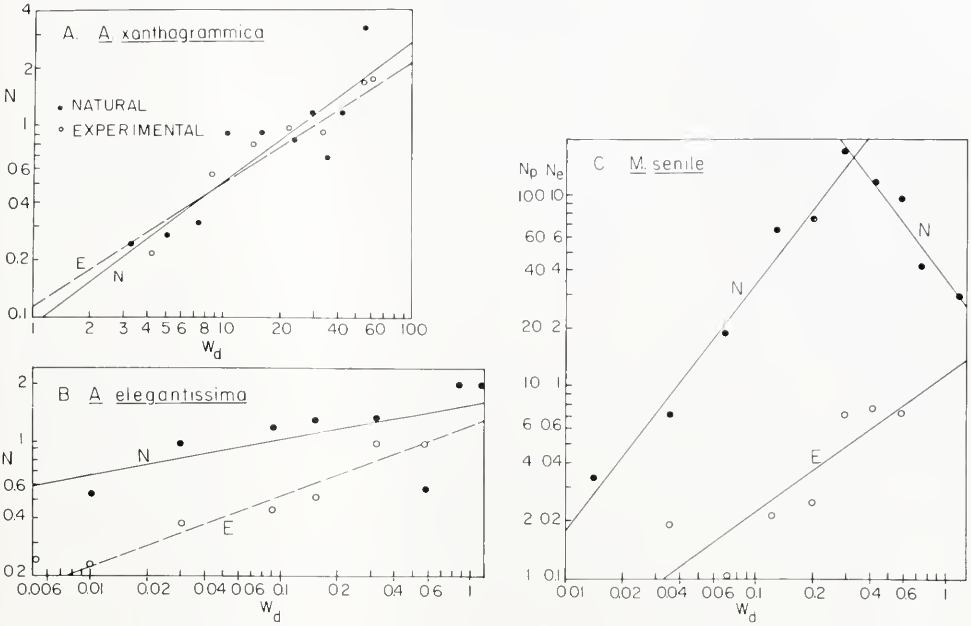

FIGURE 5. Numbers of experimental and natural prey captured in the field. Regression lines and coefficients of determination (R^2) calculated from double logarithmic transforms. Numbers in parentheses are 95% confidence limits for the exponent of W_d.

A. *Anthopleura xanthogrammica*, number of prey captured per individual (N_e experimental (E), N_p natural (N)) versus mean dry weight of each anemone size class (W_d grams).

$N_e = 0.11\ W_d^{0.67}$, (0.47, 0.88), N = 200 anemones, 7 size classes, 171 prey, $R^2 = 0.93$
$N_p = 0.0095\ W_d^{0.73}$, (0.40, 1.06), (N = 481 anemones, 10 size classes, 177 prey), $R^2 = 0.77$

B. *Anthopleura elegantissima*, number of prey captured per individual versus mean dry weight of each size class.

$N_e = 1.24\ W_d^{0.36}$, (−0.06, 0.42), (N = 112 anemones, 8 size classes, 29 prey), $R^2 = 0.95$
$N_p = 1.57\ W_d^{0.18}$, (0.24, 0.48), (N = 112 anemones, 8 size classes, 113 prey), $R^2 = 0.35$

C. *Metridium senile*, number of prey captured per individual versus mean dry weight of each size class.

$N_e = 1.17\ W_d^{0.70}$, (0.23, 1.19), (N = 107 anemones, 7 size classes, 16 prey), $R^2 = 0.74$
$N_p = 9.97\ W_d^{0.59}$, (0.07, 1.11), (N = 107 anemones, 10 size classes, 538 prey), $R^2 = 0.46$

For the first six size classes, $N_p = 61.688\ W_d^{1.19}$, (0.79, 1.59), $R^2 = 0.94$

For the last five size classes, $N_p = 0.0094\ W_d^{-1.65}$, (−2.23, −1.06), $R^2 = 0.96$

five prey for each size class of *A. xanthogrammica* up to 10 cm diameter (prey length (cm) = 0.97 anemone diameter − 2.3, $R^2 = 0.85$, N = 30) shows that maximum prey size increases with *A. xanthogrammica* diameter up to about 10 cm basal diameter (Fig. 7). Through this range, the largest prey taken are approximately equal in length to basal diameter of the anemone.

Figure 8 shows prey size distributions for all three species and for large solitary *A. elegantissima* (≧6 cm diameter). The large solitary *A. elegantissima* preys on items (mostly mussels) larger than do the small clonal individuals. Mussels constitute the major part of *A. xanthogrammica's* diet (Table I) and the size distribution of mussels captured is quite comparable to the size distribution of mussels

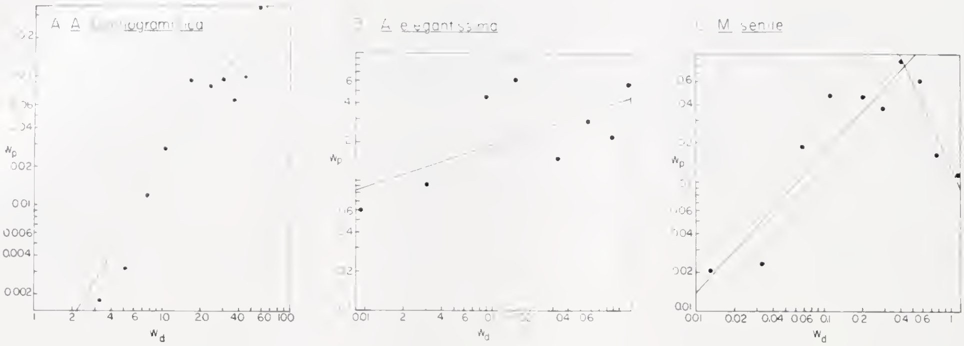

FIGURE 6. Weight of naturally occurring prey (W_p) in coelenteron contents plotted against mean individual dry weight (W_d, grams) for each size class of anemone. Regression lines and coefficients of determination (R^2) calculated from double logarithmic transforms. Numbers in parentheses are 95% confidence limits for the exponent of W_d.

A. *A. xanthogrammica*, W_p is dry weight (g) of mussel tissue $W_p = 0.00032\ W_d^{1.65}$, (1.20, 2.09), (N = 481 anemones, 10 size classes, 177 prey) $R^2 = 0.90$

B. *A. elegantissima*, W_p is wet weight (mg) of prey calculated from volume estimates, $W_p = 4.14\ W_d^{0.33}$, (−0.05, 0.71) (N = 112 anemones, 8 size classes, 113 prey) $R^2 = 0.42$

C. *M. senile*, W_p is wet weight (mg) of prey calculated from volume estimates, $W_p = 0.469\ W_d^{0.54}$, (−0.09, 1.16) (N = 107 anemones, 10 size classes, 538 prey) $R^2 = 0.86$

For the first seven size classes, $W_p = 2.66\ W_d^{1.16}$, (0.66, 1.66), $R^2 = 0.88$

For the last four size classes, $W_p = 0.109\ W_d^{-2.56}$, (−4.30, −0.84), $R^2 = 0.95$

in beds adjacent to the anemones on Tatoosh Island (mussel sizes from T. Suchanek, unpublished data).

DISCUSSION

Sea anemones capture prey by three somewhat distinct methods. First, prey suspended in the water column, usually zooplankton, are intercepted by one or more tentacles and the tentacles transfer prey to the mouth (Sebens, 1976; Purcell, 1977a). Second, sessile prey dislodged by wave action or by foraging predators are washed into the tentacle crown (Dayton, 1973; Sebens and Paine, 1978). Third, motile prey blunder into the anemone's tentacles. Sea urchins (Dayton, 1973; Dayton *et al.*, 1974; Sebens, 1976), crabs (Sebens and Laakso, 1977), and fishes (Sebens and Laakso, 1977; Hamner and Dunn, 1980) are captured in this third manner. Turbid or turbulent water conditions may facilitate this type of capture. When large prey are captured, the entire oral disc closes around the prey, and the marginal sphincter muscle contracts trapping the prey while it is being engulfed by the mouth.

Metridium senile feeds only by the first method. Its fluted oral disc and extensible column allow it to position the tentacle crown near or far from the substratum, adjusting the current velocity experienced (Koehl, 1976, 1977). Robbins and Shick (1980) showed that *M. senile* capture more prey as current velocity increases but that there is an upper level of velocity where large individuals collapse and probably cannot feed.

Anthopleura elegantissima feeds by at least the first two methods, since both zooplankton and sessile invertebrate prey occur in coelenteron contents. Single tentacles capture zooplankton and transfer them to the mouth. Oral disc closure is used for larger prey.

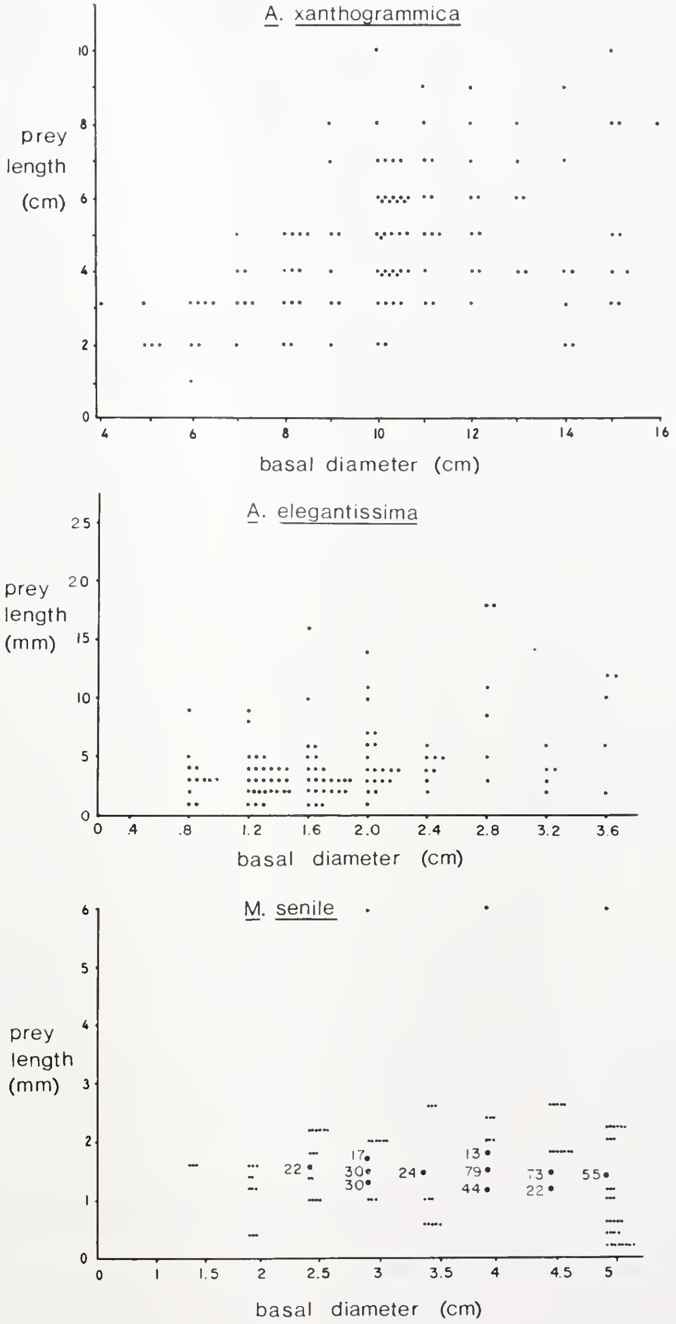

FIGURE 7. Lengths of naturally occurring prey items in anemone coelenteron contents; by basal diameter (D).

A. *A. xanthogrammica*, R = 0.44, $P < 0.01$, N = 481 anemones, 122 prey items).

B. *A. elegantissima*, R = 0.09, $P > 0.05$ (N = 112 anemones, 113 prey).

C. *M. senile*, R = 0.08, $P > 0.05$ (N = 107 anemones, 538 prey). Large numbered dots represent that number of overlapping points.

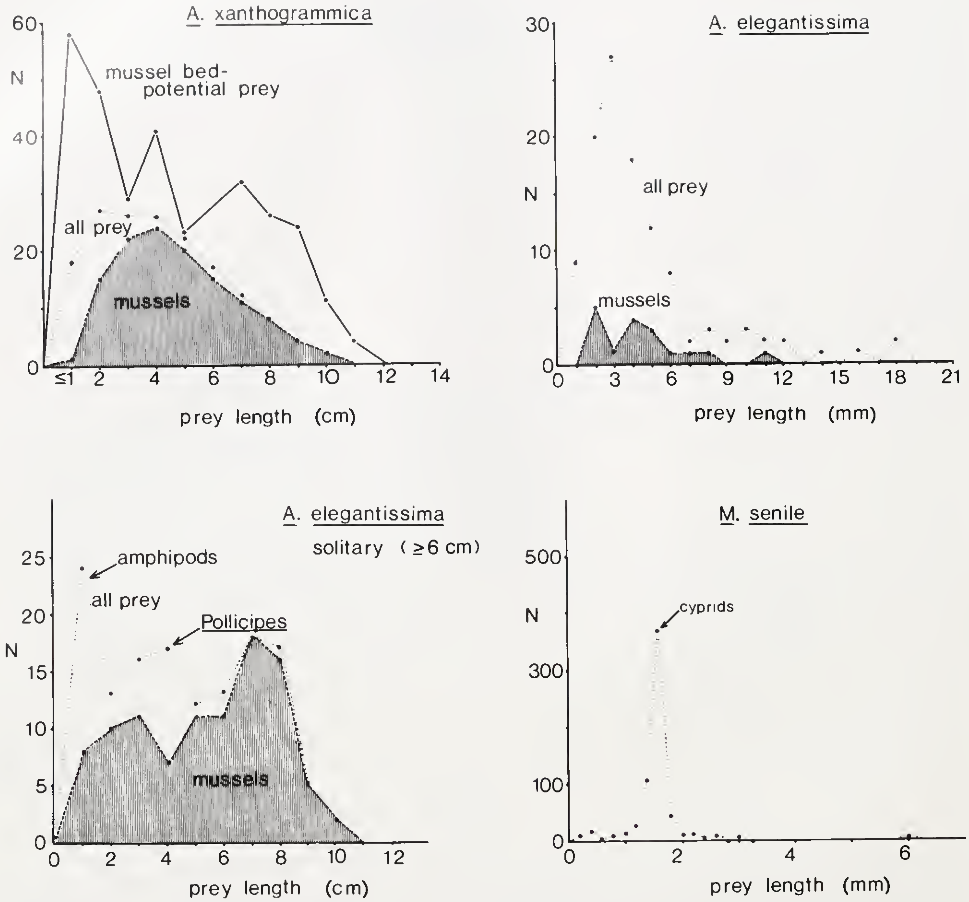

FIGURE 8. Size distribution of prey in coelenteron content samples. Mussels captured by *A. xanthogrammica* (mean length ($\bar{L}$) = 5.1 ± 2.2 cm SD, N = 481 anemones, 122 prey), mussels in the adjacent mussel bed ($\bar{L}$ = 4.6 ± 2.9 cm SD, N = 316 mussels), total prey items captured ($\bar{L}$ = 4.3 ± 2.3 cm SD, N = 481 anemones, 177 prey), prey captured by large solitary *A. elegantissima* in California ($\bar{L}$ = 4.6 ± 2.6 cm SD, N = 330 anemones, 136 prey, $\bar{L}$ = 5.4 ± 2.5 cm SD for mussel prey only, N = 99 prey), prey captured by clonal *A. elegantissima* (macroinvertebrates and zooplankters ($\bar{L}$ = 4.5 ± 3.4 mm SD, N = 112 anemones, 113 prey), *M. senile* prey (mostly zooplankters such as barnacle cyprids, copepods) $\bar{L}$ = 1.5 ± 0.4 mm SD, N = 107 anemones, 538 prey.

Anthopleura xanthogrammica captures mussels, barnacles, and other invertebrates dislodged by wave action and by asteroid foraging (Dayton, 1973) as well as crabs, gastropods, and other motile prey (Table I). Zooplankton capture by adult *A. xanthogrammica* was not observed.

Probability of prey contact for the three species of sea anemones depends on the projected surface area of the tentacle crown. The tentacles may act as filter elements to intercept zooplankton carried by currents (*M. senile, A. elegantissima*) (Rubenstein and Koehl, 1977) or as a target for intertidal prey dislodged by wave action and by predator activity (*A. xanthogrammica, A. elegantissima*) (Dayton, 1973; Sebens, 1977, in press). Anemone growth is close to isometric in *A. xan-*

thogrammica and in *M. senile*; their feeding surface areas increase as the 0.74 and 0.72 power of weight, respectively. In *A. elegantissima,* feeding surface increases more slowly, as the 0.43 power of weight. None of the three anemones amplifies its projected surface area as it grows. *M. senile,* however, adds tentacles with increasing size, such that tentacle number is a 0.84 power of weight. This allometric increase cannot change the volume of water passing the oral disc, but may be needed for efficient capture and retention of prey items. However, *M. senile* is unable to capture and hold large invertebrates such as mussels.

Capture of prey items increased as a lesser power of weight than did energetic cost for all three anemone species. This contrasts with some studies of actively pumping suspension feeders (McLusky, 1973; Bayne *et al.*, 1976; Newell *et al.*, 1977, Randløv and Riisgard, 1979; Griffiths and King, 1979) but agrees with others (Vahl, 1972, 1973; Bayne *et al.*, 1977). If feeding surfaces are not amplified allometrically during growth, passive suspension feeders have no way to compensate for their decreasing surface-to-volume ratio.

Benthic marine invertebrates often have habitat-dependent body size and indeterminate growth (Paine, 1965, 1969, 1976; Ebert, 1968; Kohn, 1971; Bertness, 1977; Sebens, 1977a, in press). Maximum size appears to depend on the energetic characteristics of a particular habitat (*e.g.* physiological stress and food availability). Transplanted individuals eventually reach a size near the mean for adults in that habitat and fluctuate around that size (*e.g.* seastars, Paine, 1976). Such patterns indicate that size may be set by the difference between local energy intake and cost. The body size at which this difference is maximized will decrease as prey availability declines, energetic cost increases, or both occur simultaneously (Sebens, 1977a, 1979, in press). It is difficult, however, to predict this optimum size directly without data on prey capture, assimilation, and metabolic cost for individuals of all sizes throughout the year (Sebens, in press).

Optimum body size of the three anemone species should be where the difference between intake and cost is at a maximum (Sebens, 1977a, 1979, in press). This difference is the energy available for gonad production summed over the entire season. It is thus directly related to fitness (reproductive success).

The energy balance can be further complicated by changes in prey size as predator size increases. Predators could potentially drop small prey from their diet or add larger prey as they grow. Neither *A. elegantissima* nor *M. senile* changed prey size as they got larger, but *A. xanthogrammica* did so quite dramatically. Large specimens of *A. xanthogrammica* in the study area ate mainly mussels (*Mytilus californianus* and *M. edulis*) although other intertidal invertebrates also occurred in the diet (Table I). As they grow, *A. xanthogrammica* are able to capture larger mussels. Thus prey capture (measured as weight per unit time) increases as a greater power of body size than does metabolic cost. Once all prey size categories can be captured, prey intake increases as does feeding surface area, since capture depends solely on the chance that a dislodged prey item is intercepted.

Anthopleura elegantissima divides by longitudinal fission, individuals dividing less often than once per year (Sebens, 1977a, 1980). Formation of these clonal aggregations creates the potential for growth to continue indefinitely. If the entire clone is considered energetically, adding new individuals by fission increases clone feeding surface and metabolic cost linearly, as functions of biomass. There is no optimum size for the aggregate, although there may be one for individual units within the clone (Fig. 10) (Sebens, 1979). Clone formation is common among sea anemones (Stephenson, 1929; Chia, 1976; Minasian, 1976; Minasian and Mariscal, 1979; Smith and Lenhoff, 1976; Johnson and Shick, 1977; Sebens, 1977a, 1979, 1980), and probably increases feeding-surface-to-biomass ratios, helping to capture relatively small prey efficiently (Sebens, 1979).

Anthopleura xanthogrammica never reproduces asexually, nor does subtidal *Metridium* (possibly a new species; Bucklin and Hedgecock, in press) although small specimens of *M. senile* in the low intertidal produce pedal lacerates frequently (Hand, 1955b; Purcell, 1977a; Hoffman, 1977; Bucklin and Hedgecock, in press). Division of *A. xanthogrammica* individuals would usually produce individuals too small to use the large mussels that make up much of their diet. Large subtidal *M. senile* anemones are crowded in dense conspecific assemblages where individual height may be important to bring the tentacle crown into currents carrying zooplankton. Thus, where vertical relief is competitively important, or where large size is necessary to capture large prey, fission may be energetically disadvantageous despite its allometric benefits.

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LITERATURE CITED

Alexander, R. M. 1971. *Size and shape.* Edward Arnold Publishing Co., London. 59 pp.

Bayne, B. L., R. J. Thompson, and J. Widdows. 1976. Physiology I. Pp. 121–265 in B. Bayne, Ed., *Marine mussels,* International Biological Programme Vol. 10, Cambridge University Press, Cambridge, Massachusetts.

Bayne, B. L., J. Widdows, and R. I. E. Newell. 1977. Physiological measurements on estuarine bivalve molluscs in the field. Pp. 57–68 in B. F. Keegan, P. O. Ceidigh, and P. J. S. Boaden, Eds., *Biology of benthic organisms.* Proc. of the 12th European Symposium on Marine Biology, Pergamon, Oxford.

Beattie, C. W. 1971. Respiratory adjustments of an estuarine coelenterate to abnormal levels of environmental phosphate and oxygen. *Comp. Biochem. Physiol.* **40B:** 907–916.

Bertness, M. D. 1977. Behavioral and ecological aspects of shore-level size gradients in *Thais lamellosa* and *Thais emarginata. Ecology* **58:** 86–97.

Brafield, A. E., and G. Chapman. 1965. The oxygen consumption of *Pennatula rubra* Ellis and some other anthozoans. *Z. Vergl. Physiol.* **50:** 363–370.

BUCKLIN, A., AND D. HEDGECOCK. In press. Biochemical genetic evidence for a third species of *Metridium* (Coelenterata, Actiniaria). *Mar. Biol.*

CHIA, F. S. 1976. Sea anemone reproduction: patterns and adaptive radiations. Pp. 261–270 in G. O. Mackie, Ed., *Coelenterate ecology and behavior.* Plenum Press, New York.

DAYTON, P. K. 1973. Two cases of resource partitioning in an intertidal community: making the right prediction for the wrong reason. *Am. Nat.* **107:** 662–670.

DAYTON, P. K., G. A. ROBILLIARD, R. T. PAINE, AND L. B. DAYTON. 1974. Biological accomodation in the benthic community at McMurdo Sound, Antarctica. *Ecol. Monogr.* **44:** 105–128.

EBERT, T. A. 1968. Growth rates of the sea urchin *Stronglyocentrotus purpuratus* related to food availability and spine abrasion. *Ecology* **49:** 1075–1093.

FORD, C. E. 1964. Reproduction in the aggregating sea anemone *Anthopleura elegantissima. Pac. Sci.* **18:** 138–145.

FOX, D. L., AND W. R. COE. 1943. Biology of the California sea mussel, *Mytilus Californianus. J. Exp. Zool.* **93:** 205–249.

FRANCIS, L. 1973a. Clone specific segregation in the sea anemone *Anthropleura elegantissima. Biol. Bull.* **144:** 64–72.

FRANCIS, L. 1973b. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related anemones. *Biol. Bull.* **144:** 73–92.

FRANCIS, L. 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima. Biol. Bull.* **150:** 361–376.

FRANCIS, L. 1979. Contrast between solitary and clonal lifestyles in the sea anemone *Anthopleura elegantissima. Am. Zool.* **19:** 669–681.

GOULD, S. J. 1966. Allometry and size in ontogeny and phylogeny. *Biol. Rev.* **41:** 587–640.

GRIFFITHS, C. L., AND J. A. KING. 1979. Some relationships between size, food availability and energy balance in the ribbed mussel *Aulacomya ater. Mar. Biol.* **51:** 141–149.

HAMNER, W. M., AND D. F. DUNN. 1980. Tropical corallimorpharia (Coelenterata: Anthozoa): feeding by envelopment. *Micronesica* **16:** 37–42.

HAND, C. 1955a. The sea anemones of central California. II, The endomyarian and mesomyarian anemones. *Wassmann J. Biol.* **13:** 37–99.

HAND, C. 1955b. The sea anemones of central California. III. The acontiarian anemones. *Wassmann J. Biol.* **13:** 189–251.

HOFFMANN, R. 1977. Genetics and asexual reproduction of the sea anemone *Metridium senile. Biol. Bull.* **151:** 478–488.

HOLTER, H., AND E. ZEUTHEN. 1948. Metabolism and reduced weight in starving *Chaos chaos* (L.). *C. R. Lab. Carlsberg Ser. Chim.* **26:** 277–296.

JENNISON, B. L. 1979. Gametogenesis and reproductive cycles in the sea anemone *Anthopleura elegantissima* (Brandt, 1835). *Can. J. Zool.* **57:** 403–411.

JOHNSON, L. L. AND J. M. SHICK. 1977. Effects of fluctuating temperature and immersion on asexual reproduction in the intertidal sea anemone *Haliplanella luciae* (Verrill) in laboratory culture. *J. Exp. Mar. Biol. Ecol.* **28:** 141–149.

JONES, R. 1976. Growth of fishes. Pp. 251–279. in D. H. Cushing and J. J. Walsh, Eds. *The ecology of the seas,* Blackwell Scientific Publications, Oxford.

JØRGENSON, C. G. 1975. Comparative physiology of suspension feeding. *Ann. Rev. Physiol.* **37:** 57–79.

KITCHELL, J. F., D. J. STEWART, AND D. WEININGER. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and Walleye (*Stizostedion vitreum vitreum*). *J. Fish. Res. Board Can.* **34:** 1922–1935.

KITCHELL, J. F., W. H. NEAL, A. E. DIZON, AND J. J. MAGNUSON. 1978. Bioenergetic spectra of skipjack and yellowfin tunas. Pp. 357–369. in G. Sharp and A. Dizon, Eds., *The physiological ecology of tunas.* Academic Press, New York.

KLEIBER, M. 1932. Body size and metabolism. *Hilgardia* **6:** 315–353.

KOEHL, M. A. R. 1976. Mechanical design in sea anemones. Pp. 23–31 in G. O. Mackie, Ed., *Coelenterate ecology and behavior.* Plenum Press, New York.

KOEHL, M. A. R. 1977. Effects of sea anemones on the flow forces they encounter. *J. Exp. Biol.* **69:** 87–106.

KOHN, A. J. 1971. Diversity, utilization of resources, and adaptive radiation in shallow-water marine invertebrates of tropical oceanic islands. *Limnol. Oceanogr.* **16:** 332–348.

MCLUSKY, D. S. 1973. The effect of temperature on the oxygen consumption and filtration rates of *Chlamys* (*Aequipecten*) *opercularis* (L.) (Bivalvia). *Ophelia* **10:** 141–154.

MCMAHON, T. 1973. Size and shape in biology. *Science* **179:** 1201–1204.

MINASIAN, L. L. 1976. Characteristics of asexual reproduction in the sea anemone *Haliplanella luciae* (Verrill), reared in the laboratory. Pp. 289–298. in G. O. Mackie, Ed., *Coelenterate ecology and behavior.* Plenum Press, New York.

MINASIAN, L. L., AND R. N. MARISCAL. 1979. Characteristics and regulation of fission activity in clonal cultures of the cosmopolitan sea anemone, *Haliplanella luciae* (Verrill). *Biol. Bull.* **157:** 478-493.

MUSCATINE, L. 1961. Symbiosis in marine and freshwater coelenterates. Pp. 255-268 in H. M. Lenhoff and W. F. Loomis, Ed., *The biology of Hydra.* University of Miami Press, Coral Gables, Florida.

NEWELL, R. C., L. G. JOHNSON, AND L. H. KOFOED. 1977. Adjustment of the components of energy balance in response to temperature change in *Ostrea edulis. Oecologia* **30:** 97-110.

PAINE, R. T. 1965. Natural history, limiting factors and energetics of the opisthobranch *Navanax inermis. Ecology* **46:** 603-619.

PAINE, R. T. 1976. Size-limited predation: an observational and experimental approach with the *Mytilus-Pisaster* interaction. *Ecology* **57:** 858-873.

PICKARD, G. L. 1975. *Descriptive physical oceanography.* Pergamon Press, Oxford. 233 pp.

PORTER, J. W. 1974. Zooplankton feeding by the Caribbean reef-building coral *Montastrea cavernosa.* Pp. 111-125 in *Proceedings of the second international coral reef symposium.* Great Barrier Reef Committee, Brisbane, Australia.

PURCELL, J. E. 1977a. The diet of large and small individuals of the sea anemone *Metridium senile. Bull. S. Cal. Acad. Sci.* **76:** 168-172.

PURCELL, J. E. 1977b. Aggressive function and induced development of catch tentacles in the sea anemone *Metridium senile* (Coelenterata, Actiniaria). *Biol. Bull.* **153:** 355-368.

RANDLØV, A., AND H. U. RIISGARD. 1979. Efficiency of particle retention and filtration rate in four species of ascidians. *Mar. Ecol. Prog. Ser.* **1:** 55-59.

REICHLE, D. E. 1967. Relation of body size to food intake, oxygen consumption, and trace element metabolism in forest floor arthropods. *Ecology* **49:** 538-541.

ROBBINS, R. E., AND J. M. SHICK. 1980. Expansion-contraction behavior in the sea anemone *Metridium senile*: environmental cues and energetic consequences. Pp. 101-116 in D. C. Smith and Y. Tiffon, Eds., *Nutrition in the lower metazoa.* Pergamon Press, New York.

RUBENSTEIN, D. I., AND M. A. R. KOEHL. 1977. The mechanisms of filter-feeding: some theoretical considerations. Am. Nat. **111:** 981-994.

SASSAMAN, C., AND C. P. MANGUM. 1973. Relationship between aerobic and anaerobic metabolism in estuarine anemones. *Comp. Biochem. and Physiol.* **44A:** 1313-1319.

SASSAMAN, C., AND C. P. MANGUM. 1974. Gas exchange in a cerianthid. *Exp. Zool.* **188:** 297-306.

SCHMIDT-NIELSON, K. 1974. Scaling in biology: the consequences of size. *Exp. Zool.* **194:** 287-308.

SEBENS, K. P. 1976. The ecology of Caribbean sea anemones in Panama: utilization of space on a coral reef. Pp. 67-78 in G. O. Mackie, Ed., *Coelenterate ecology and behavior.* Plenum Press, New York.

SEBENS, K. P. 1977a. Habitat suitability, reproductive ecology, and the plasticity of body size in two sea anemone populations (*Anthropleura elegantissima* and *A. xanthogrammica*). Ph.D. thesis, University of Washington, Seattle. 258 pp.

SEBENS, K. P., 1977b. Autotrophic and heterotrophic nutrition of coral reef zoanthids. Pp. 397-406 in *Proceedings of the third international coral reef symposium,* I. Committee on Coral Reefs, Miami.

SEBENS, K. P. 1979. The energetics of asexual reproduction and colony formation in benthic marine invertebrates. *Am. Zool.* **19:** 683-697.

SEBENS, K. P. 1980. The control of asexual reproduction and indeterminate body size in the sea anemone, *Anthopleura elegantissima* (Brandt). *Biol. Bull.* **158:** 370-382.

SEBENS, K. P. In press. The limits to indeterminate growth: an optimal size model applied to passive suspension feeders. *Ecology.*

SEBENS, K. P., AND G. LAAKSO. 1977. The genus *Tealia* (Anthozoa Actiniaria) in the waters of the San Juan Archipelago and the Olympic Peninsula. *Wasmann J. Biol.* **35:** 152-168.

SEBENS, K. P., AND R. T. PAINE, 1978. The biogeography of anthozoans along the west coast of South America. Pp. 219-237 in Proceedings of the International Symposium on Marine Biogeography and Ecology of the Southern Hemisphere. Vol. I. *New Zealand Dept. of Scientific and Industrial Research Inf. Series* 137.

SHICK, J. M., AND W. I. BROWN. 1977. Zooxanthellae-produced O_2 promotes sea anemone expansion and eliminates oxygen debt under environmental hypoxia. *Exp. Biol.* **201:** 149-155.

SHICK, J. M., W. I. BROWN, E. G. DOLLIVER, AND S. R. KAYAR. 1979. Oxygen uptake in sea anemones: effects of expansion, contraction and exposure to air and the limitations of diffusion. *Physiol. Zool.* **52:** 50-61.

SHICK, J. M., AND R. J. HOFFMAN. 1980. Effects of the trophic and physical environments on asexual reproduction and body size in the sea anemone *Metridium senile.* Pp. 211-216 in P. Tardent and R. Tardent, Eds., *Developmental and cellular biology of coelenterates,* Elsevier, North-Holland Biomedical Press.

Smith, N., III, and H. M. Lenhoff. 1976. Regulation of frequency of pedal laceration in a sea anemone. Pp. 117–126 in G. O. Mackie, Ed., *Coelenterate ecology and behavior,* Plenum Press, New York.

Sokal, R. R. and J. F. Rohlf. 1969. *Biometry: The principles and practice of statistics in biological research.* W. H. Freeman and Co., San Francisco. 776 pp.

Stephenson, T. A. 1929. On methods of reproduction as specific characters. *J. Mar. Biol. Assoc. U. K.* **16:** 131–172.

Thompson, D. W. 1917. *On growth and form.* Cambridge University Press, London.

Thompson, R. J. and B. L. Bayne. 1979. Some relationships between growth, metabolism and food in the mussel *Mytilus edulis. Mar. Biol.* **27:** 317–326.

Vadas, R. L. 1977. Preferential feeding: an optimization strategy in sea urchins. *Ecol. Monogr.* **47:** 337–371.

Vahl, O. 1972. Particle retention and relation between water transport and oxygen uptake in *Chlamys opercularis* (L.) (Bivalvia). *Ophelia* **10:** 67–74.

Vahl, O. 1973. Pumping and oxygen consumption rates of *Mytilus edulis* L. of different sizes. *Ophelia* **12:** 45–52.

Warren, C. E., and G. E. Davis. 1967. Laboratory studies on the feeding, bioenergetics and growth of fish. Pp. 175–214 in S. D. Gerking, Ed., *The biological basis of fresh water fish production,* Blackwell Scientific Publications, Oxford.

Widdows, J. V. 1978. Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis. J. Mar. Biol. Assoc. U. K.* **58:** 109–124.

Winter, J. E. 1969. Uber den Einflub der Nahrungskonzentration und anderer Faktoren auf Filtrierleistung und Nahrungsausnutzung der Muscheln *Artica islandica* und *Modiolus modiolus. Mar. Biol.* **4:** 87–135.

Winter, J. E. 1973. The filtration rate of *Mytilus edulis* and its dependence of algal concentration, measured by a continuous automatic recording apparatus. *Mar. Biol.* **22:** 317–328.

Winter, J. E. 1978. A review of the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture* **13:** 1–33.

Zeuthen, E. 1948a. Body size and metabolic rate in the animal kingdom with special regard to the marine microfauna. *C. R. Lab. Carlsberg Ser. Chim.* **26:** 17–161.

Zeuthen, E. 1948b. Reduced weight and volume during starvation of amoeba *Chaos chaos* (L.) *C. R. Lab. Carlsberg Ser. Chim.* **26:** 267–276.

Zeuthen, E. 1953. Oxygen uptake as related to body size in organisms. *Q. Rev. Biol.* **28:** 1–12.

Reference: *Biol. Bull.* **161**: 172–179. (August, 1981)

MECHANISM OF STARFISH SPAWNING. IV. TENSION GENERATION IN THE OVARIAN WALL BY 1-METHYLADENINE AT THE TIME OF SPAWNING

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ABSTRACT

Tension generation in the starfish ovaries at the time of spawning was investigated using an isometric tensiometer. Ovarian fragments containing oocytes gradually generated tension after 1-methyladenine application; before 1-methyladenine application the ovarian walls were not under strong tension. Isolated ovarian walls did not generate tension after 1-methyladenine treatment, but did after the application of jelly substance. As the hormone 1-methyladenine is known to act on oocytes surrounded by follicle envelopes to induce germinal vesicle breakdown, which inevitably results in the breakdown of follicle envelopes, it is concluded that (1) jelly substance acts directly to induce contraction of ovarian walls, (2) 1-methyladenine acts indirectly, contraction being caused as a result of the hormone's action on breakdown of germinal vesicles and follicle envelopes, after which jelly substance contacts the ovarian wall.

INTRODUCTION

Starfish spawning, including germinal vesicle breakdown and egg shedding, is caused by gonad-stimulating substance (GSS) secreted from nervous tissue (Chaet and McConnaughy, 1959; Chaet and Rose, 1961; Kanatani, 1964; Chaet, 1966, 1967; Kanatani and Ohguri, 1966). Since oocytes, adherently surrounded by follicle cells before spawning, are forced by contraction of ovarian walls through narrow gonopores, oocyte separation from follicle cells and contraction of ovarian walls are essential in shedding. Previous work has shown that GSS itself neither induces contractions in ovarian walls nor separates oocytes from follicles. On the other hand, 1-methyladenine (1-MeAde, produced by follicle cells under the influence of GSS) can cause oocyte-follicle separation following germinal vesicle breakdown. The chemical, a natural trigger of germinal vesicle breakdown, can by itself cause ovaries to spawn (Kanatani and Shirai, 1967; Schuetz and Biggers, 1967; Kanatani, 1967, 1969; Kanatani *et al.*, 1969; Schuetz, 1969; Hirai and Kanatani, 1971; Hirai *et al.*, 1973).

However, no work has dealt with contraction-inducing substance(s) within the ovary. The present study was carried out to examine whether 1-MeAde has contraction-inducing activity not, and how the ovarian wall contracts at spawning. We used an isometric tensiometer to measure contraction of the ovarian wall. The results showed that 1-MeAde causes contraction in ovarian fragments with oocytes, but not in isolated ovarian walls without oocytes. Isolated-ovarian-wall contraction

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Abbreviations: GSS: gonad-stimulating substance; 1-MeAde: 1-methyladenine; JF: jelly fraction.

was induced by jelly substance. The reason why eggs are shed concurrent with germinal vesicle breakdown in starfish under natural conditions is discussed.

MATERIALS AND METHODS

Starfish (*Asterina pectinifera*) were collected in May in Kanagawa prefecture and kept in laboratoy aquaria with circulating cold seawater at the National Institute for Basic Biology. Ovaries were cut and washed with seawater. One branch of the ovary (about 5 mm long) was used in each measurement. Isolated ovarian walls without oocytes were prepared from ovarian fragments by cutting along their longitudinal axes with fine scissors and removing oocytes with follicle cells using fine forceps.

Artificial seawater was used (Jamarin or Calcium-free Jamarin, Jamarin Laboratory, Osaka). Jelly substance was obtained by dissolving jelly coats with HCl (pH 5.5) using eggs previously collected by treating ovaries for 1 h with 10^{-6} *M* 1-MeAde (Sigma). The egg suspension was kept for 1 h and occasionally agitated gently. The supernatant, containing dissolved jelly, was then centrifuged to remove debris and adjusted to pH 8 with NaOH. Concentrated jelly solution, obtained by centrifugation using Centri-flo C-25, was designated "jelly fraction" (JF). This process concentrated only molecules with molecular weights of more than about 25,000, and did not concentrate salt components. By this technique, about 1/100 of the volume of intact jelly was concentrated in JF (calculating the volume of intact jelly by measuring egg diameter, jelly coat thickness, and number of eggs).

Isometric contraction of ovarian fragments was measured with a horizontal-type tensiometer having a sensitivity of about 0.1 mg (Fig. 1) (Kamiya *et al.*, 1972; Yoshimoto and Kamiya, 1978). This enabled us to measure tension generated by a specimen submerged in a small quantity of solution (about 1 ml). A specimen was connected to the two fine glass rods of the tensiometer with a surgical adhesive agent, Aron alpha A (alkyl-α-cyanoacrylate, Sankyo Co.). Specimens were attached to the rods in the air (completed within 1 min). After attachment, the specimens were immersed in the appropriate test solution.

RESULTS

Ovarian fragments with oocytes

Table I (upper part) shows that intact ovarian fragments, fixed on glass rods and treated with 1-MeAde, generated between 20 and 82 mg tension (mean 45 mg). Figure 2 shows a typical example.

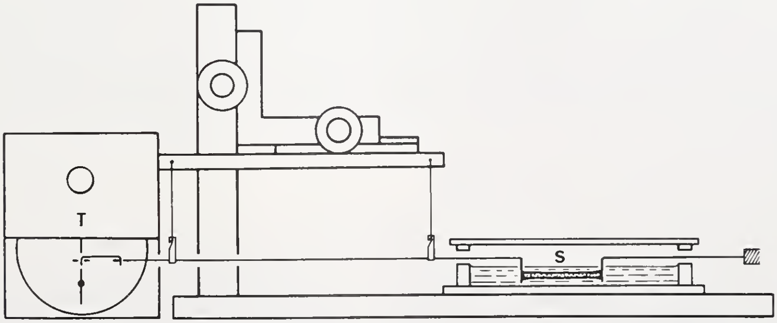

FIGURE 1. Scheme of a horizontal-type tensiometer. S, Specimen; T, Tension transducer.

TABLE I

Induction of tension in ovarian fragments. SE = standard error.

Exp. No.	Time lag (min)	Retained tension after pulling (mg)	Tension generation (mg)	Small oscillation	
				Amplitude (mg)	Period (s)
Intact 1	11	—	40	±1	60
2	7	—	57	±1	60
Fragments 3	8	—	20	±1	60
4	18	—	27	±1	80
5	9	—	82	±1	48
Mean ± SE	11 ± 2	—	45 ± 11	±1	62 ± 5
Incised 1	15	6	125	±2	48
2	16	15	130	±2	53
3	16	6	80	±2	96
Fragments 4	16	9	105	±5	60
5	5	9	91	±3	24
6	10	13	73	±1	60
Mean ± SE	13 ± 2	10 ± 2	101 ± 10	±2.5	57 ± 10

After a time lag (between 1-MeAde application [final 10^{-5} M] to the beginning of tension increase) of mean 11 min, tension began to increase, reaching a high plateau 50–60 min after 1-MeAde application (21°C–23°C). This level was maintained for at least 2 h. Discharge of eggs from a cut end of the ovarian fragment usually began during the ascent to the high plateau. In addition to the tension generation, two types of oscillations were observed. One is designated small oscillation, with a period of about 1 min, and the other large oscillation, with a period of about 15 min (Fig. 2).

Next, ovarian fragments were simply cut along their longitudinal axes, leaving most oocytes still adhering to the walls. Since such incised fragments shortened from about 5 mm to 4.6 mm after cutting without 1-MeAde application, they were pulled to their original length by controlling the glass rod after the fragments were set on the tensiometer. Tension was generated just after this pulling, from several to more than a hundred 10 mgs, but then decreased rapidly (about 10 mg/min) and stayed at low levels (just over 10 mg) as shown in Table I (retained tension after pulling). After the fragments reached this resting state (about 20 min after pulling), 1-MeAde was added at a final concentration of 10^{-5} M, much as with intact ovarian fragments. As shown by Table I (lower part), the treated fragments generated strong tension (mean 101 mg) after a time lag (mean 13 min). Small oscillations were more obvious than in intact fragments, but large oscillations were not observed.

Contraction of isolated ovarian walls

Isolated ovarian walls without oocytes, prepared from intact fragments (not treated with 1-MeAde), were connected to the tensiometer and pulled, as were simply incised ones. As shown in Table II (upper part), they did not generate tension after 1-MeAde application, though 1-MeAde usually induced small oscillations. As these specimens generated sudden, temporary (but not retained) tension

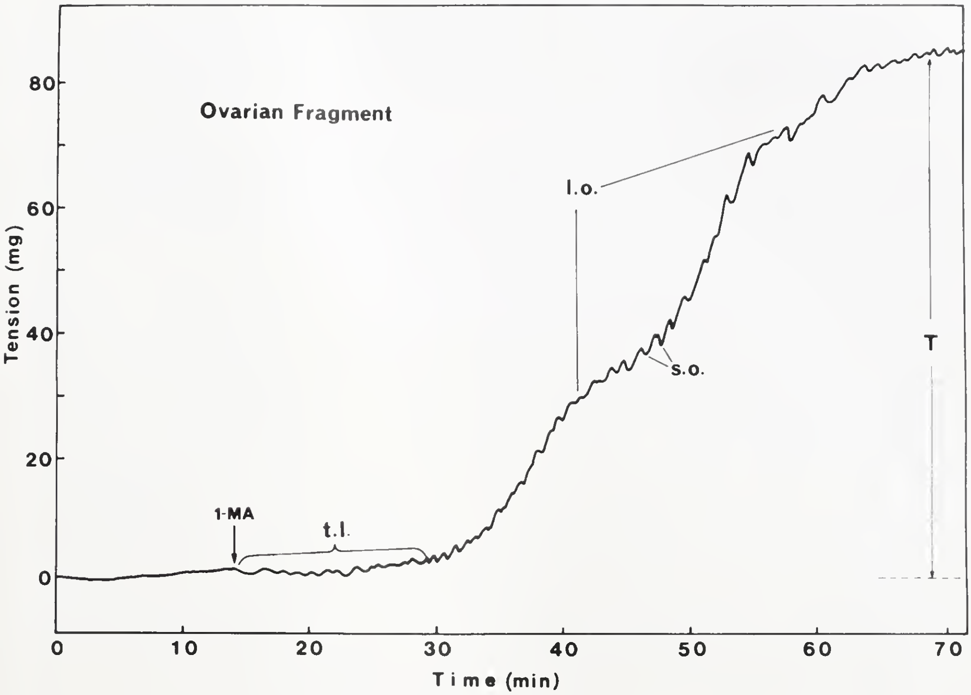

FIGURE 2. Induction of tension generation in intact ovarian fragments after 1-MeAde application. 1-MA, 1-MeAde application; T, tension generated; s.o., small oscillation in tension change; l.o., large oscillation in tension change; t.l., time lag.

on addition of KCl solution (10/9 *M*, one drop applied close to the specimen), the contractile structure had not been harmed during preparation. Figure 3 (upper left insertion) shows the tension change in specimens after application of KCl solution.

When isolated ovarian walls prepared from 10^{-5} *M* 1-MeAde treated (15 min) fragments were connected to the tensiometer, the retained tensions stayed at high levels (mean 27 mg) in the presence of 1-MeAde. This indicates that contraction actually had begun in ovarian walls treated with 1-MeAde.

Since the important difference between ovarian fragments and isolated walls was the presence or absence of oocytes, some intermediate substance(s) from oocytes seem to be involved in inducing contraction. We tested jelly substance, because jelly surrounds every oocyte and inevitably contacts the ovarian wall after breakdown of follicle envelopes from oocytes. The lower part of Table II shows that isolated walls generated tension after adding JF, with a mean time lag of 2 min (in this case the time lag was regarded as the period between JF application and beginning of tension increase). Small oscillations were much smaller than with 1-MeAde. (Fig. 3).

DISCUSSION

It was initially thought that the ovarian walls of intact fragments were originally under strong tension, corresponding to that generated in fragments to which 1-MeAde had been applied, because after incision the ovaries turned inside-out, with

TABLE II

Tension retention and generation in isolated ovarian walls.

Exp. No.	Retained tension after pulling (mg)	Time lag (min)	Tension generation (mg)	Small oscillation	
				Amplitude (mg)	Period (s)
1-MeAde application					
1	10	—	0	±1	80
2	20	—	0	±1	96
3	11	—	0	±0	irregular
4	25	—	0	±2	40
5	10	—	0	±0	irregular
Mean ± S.E.	15 ± 3	—	0	±1	72 ± 17
JF application					
1	9	2	35*	not detectable	
2	18	2	28*	not detectable	
3	9	2	29	not detectable	
4	22	2	27	not detectable	
5	11	3	18	not detectable	
6	10	2	21	not detectable	
Mean ± S.E.	13 ± 2	2 ± 0.2	26 ± 2	not detectable.	

* JF and 1-MeAde ($10^{-5}M$) added.

the mass of oocytes lying on the external surface, and the length of the fragment shortened (Kanatani, 1967). However, as shown in Table I (retained tension after pulling), these ovarian walls did not retain the strong tension displayed just after they were pulled. Therefore, it is unlikely that ovarian walls are originally under strong tension. However, when the incised ovarian fragments were tested without pulling (no tension displayed before 1-MeAde application), they generated less tension after 1-MeAde application than did pulled fragments. Smooth muscle generally displays a well-defined length-tension relationship: if the original length of

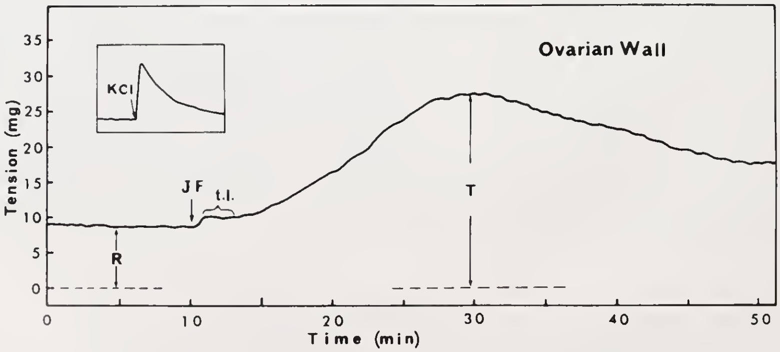

FIGURE 3. Induction of tension generation in isolated ovarian walls prepared from intact ovarian fragments after "jelly fraction" (JF) application. JF, JF application; R, Retained tension; T, tension generated; t.l., time lag; KCl, KCl application.

the specimen is increased or reduced, its ability to generate tension changes. If smooth muscle is shortened, its ability to generate tension is reduced (Millman, 1964). Thus ovarian walls at their original length are more able to generate strong tension than after shortening.

We experimented using isolated ovarian walls because the purpose of the present study was not to investigate the mechanism of smooth muscle contraction, but to test 1-MeAde action as the direct inducer of ovarian contraction. As shown in Table II, isolated walls did not generate tension after 1-MeAde application, though these specimens displayed phasic contraction after stimulation with KCl (Twarog, 1976), as described in Results. However, these specimens generated strong tension after JF was added (Table II, lower part). Therefore, it can be concluded that JF contains the substance inducing contraction of isolated ovarian walls.

Fully grown ovaries are filled with numerous oocytes with a jelly coat surrounded by follicle cells adhering to each other. At spawning, the oocytes have already lost the follicle envelopes and are freely movable, and the ovarian wall contracts. GSS (released from nervous tissue) causes ovaries to spawn within about 30 min after its application. However, GSS itself does not possess oocyte-separating activity affecting oocyte-follicle cell adhesion, or contraction-inducing activity on ovarian walls. With respect to spawning GSS only acts on follicle cells to produce 1-MeAde (Kanatani, 1973, 1979; Kanatani and Shirai, 1969, 1970). On the other hand, 1-MeAde, produced by follicle cells within a very short period after GSS application, by itself causes ovaries to spawn. GSS or 1-MeAde initiate spawning in approximately the same time, about 30 min. Moreover, 1-MeAde has oocyte-separating activity: When oocytes still surrounded by follicle cells were immersed in seawater containing 1-MeAde, germinal vesicle breakdown took place within 15–20 min and inevitably resulted in the breakdown of follicle envelopes. Although we have not yet investigated the actual process of how the follicle envelopes break down, we know that 1-MeAde acts on the separation of the oocyte from the follicle cell. This follicle envelope breakdown subsequently leads to direct contact between ovarian walls and the jelly layer of oocytes within ovaries. In summary, jelly substance acts directly on contraction of ovarian walls, while 1-MeAde acts indirectly, causing contraction as a result of the action of the hormone on germinal-vesicle and follicle-envelope breakdown, after which jelly substance contacts the ovarian wall.

Although follicle cells and oocytes can be experimentally separated in calcium-free seawater, in this condition ovarian fragments never contract, even if they contact jelly (Kanatani, 1967; Schuetz and Biggers, 1968; Kanatani and Shirai, 1969, 1970). Therefore, when jelly substance causes ovarian walls to contract, calcium must be available for certain processes, undoubtedly including the actual contraction of muscle.

Egg spawning in individuals (egg shedding from gonopores) usually occurs within 1 h after 1-MeAde injection into the coelomic cavity. Microscopic observations show that the gonopore, closed until egg discharge begins, is opened by eggs. Since the tension generated will have almost reached its high plateau by the time the gonopore opens, the force that keeps the gonopore closed (which we have not measured) must be less than that of the high plateau level in the ovarian wall. And since egg discharge continues until almost all eggs are expelled, the mechanism which keeps the force in the ovarian walls stronger than that at the gonopore is essential for the completion of spawning.

Ovarian wall contraction induced by jelly components may be a part of the mechanism which induces ovarian contraction in the limited area around oocytes which have undergone germinal vesicle breakdown: in an intact ovary during spawning, as more and more oocytes mature, the number of limited areas contracting within the ovarian wall would thus increase until the total tension was greater than the force at the gonopore. This tension would be greatest once all, or almost all oocytes were mature. Thus, only mature oocytes might be spawned.

Contraction-inducing substances common to both female and male may exist, because 1-MeAde, produced by interstitial cells of the testes (Kubota *et al.*, 1977), also causes shedding of spermatozoa after a certain time lag (less than 1 h).

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LITERATURE CITED

Chaet, A. B. 1966. Neurochemical control of gamete release in starfish. *Biol. Bull.* **130:** 43–58.

Chaet, A. B. 1967. Gamete release and shedding substance of sea-stars. *Symp. Zool. Soc. Lond.* **20:** 13–24.

Chaet, A. B., and R. A. McConnaughy. 1959. Physiologic activity of nerve extracts. *Biol. Bull.* **117:** 407–408.

Chaet, A. B., and R. A. Rose. 1961. Further studes on the gamete "shedding substance" from radial nerves. *Biol. Bull.* **121:** 385–386.

Hirai, S., and H. Kanatani. 1971. Site of production of meiosis-inducing substance in ovary of starfish. *Exp. Cell Res.* **67:** 224–227.

Hirai, S., K. Chida, and H. Kanatani. 1973. Role of follicle cells in maturation of starfish oocytes. *Dev. Growth Differ.* **15:** 21–31.

Kamiya, N., R. D. Allen, and R. Zeh. 1972. Contractile properties of the slime mold strand. *Acta Protozoologica,* **11:** 113–124.

Kanatani, H. 1964. Spawning of starfish: Action of gamete-shedding substance obtained from radial nerves. *Science* **146:** 1177–1179.

Kanatani, H. 1967. Neural substance responsible for maturation of oocytes and shedding of gametes in starfish. *Gunma Symp. Endocrinol.* **4:** 65–78.

Kanatani, H. 1969. Induction of spawning and oocyte maturation by 1-methyladenine in starfishes. *Exp. Cell Res.* **57:** 333–337.

Kanatani, H. 1973. Maturation-inducing substance in starfishes. *Int. Rev. Cytol.* **35:** 253–298.

Kanatani, H. 1979. Oogenesis: Hormonal mechanism of oocyte maturation. Pp. 3–15 in J. Ebert and T. Okada, Eds., *Mechanism of cell change.* John Wiley and Sons, Inc., New York.

Kanatani, H., and M. Ohguri. 1966. Mechanism of starfish spawning. I. Distribution of active substance responsible for maturation of oocytes and shedding of gametes. *Biol. Bull.* **131:** 104–114.

Kanatani, H., and H. Shirai. 1967. *In vitro* production of meiosis-inducing substance by nerve extract in ovary of starfish. *Nature* **216:** 284–286.

Kanatani, H., and H. Shirai. 1969. Mechanism of starfish spawning. II. Some aspects of action of neural substance obtained from radial nerves. *Biol. Bull.* **137:** 297–311.

Kanatani, H., and H. Shirai. 1970. Mechanism of starfish spawning. III. Properties and action of meiosis-inducing substance produced in gonad under influence of gonad-stimulating substance. *Dev. Growth Differ.* **12:** 119–140.

Kanatani, H, H. Shirai, K. Nakanishi, and T. Kurokawa. 1969. Isolation and identification of meiosis inducing substance in starfish. *Nature* **211:** 273–274.

Kubota, J., H. Shirai, and H. Kanatani. 1977. 1-Methyladenine-producing cell in starfish testis. *Exp. Cell Res.,* **106:** 63–70.

Millman, B. M. 1964. Contraction in the opaque part of the adductor muscle of the oyster (*Crassostrea angulata*). *J. Physiol.* **173:** 238–262.

Schuetz, A. W. 1969. Induction of oocyte shedding and meiotic maturation in *Pisaster ochraceus*:

kinetic aspects of radial nerve factor and ovarian factor induced changes. *Biol. Bull.* **137:** 524–534.

SCHUETZ, A. W., AND J. D. BIGGERS. 1967. Regulation of germinal vesicle breakdown in starfish oocytes. *Exp. Cell Res.* **46:** 624–628.

SCHUETZ, A. W., AND J. D. BIGGERS. 1968. Effect of calcium on the structure and functional response of the starfish ovary to radial nerve factor. *J. Exp. Zool.* **168:** 1–10.

TWAROG, B. M. 1976. Aspects of smooth muscle function in Molluscan catch muscle. *Physiol. Rev.* **56:** 829–838.

YOSHIMOTO, Y., AND N. KAMIYA. 1978. Studies on contraction rhythm of the plasmodial strand. IV. Site of active oscillation in an advancing plasmodium. *Protoplasma* **95:** 123–133.

Reference: *Biol. Bull.* **161**: 180–198. (August, 1981)

OBSERVATIONS ON THE SYMBIOSIS WITH ZOOXANTHELLAE AMONG THE TRIDACNIDAE (MOLLUSCA, BIVALVIA).

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ABSTRACT

The symbiosis with zooxanthellae among tridacnid bivalve molluscs has been examined in order to assess the morphological relation of the algae to the hosts' tissues and the dynamics of the interactions between the algae and the clams.

Light and electron microscopic evidence indicates that the zooxanthellae located in the haemal sinuses of the hypertrophied siphon are intercellular in all six tridacnid species studied. The algae pass from the siphonal tissues to the alimentary tract from which they are voided as feces. Many of the defecated symbionts are morphologically intact and photosynthetically functional. The concretions in the kidneys of tridacnids, previously thought to be the indigestible remains of zooxanthellae, are identified as phosphorite deposits, which are not unique to tridacnids.

Analyses of photosynthesis-radiant flux relations in *Tridacna maxima* indicate that over 24 h the algae produce more oxygen than is consumed by the association, and estimates suggest that the algae may contribute more than 50% of the animals' metabolic carbon requirements.

INTRODUCTION

Tridacnid bivalves harbor endosymbiotic dinoflagellates, commonly referred to as zooxanthellae, within the haemal sinuses of the hypertrophied siphon (Brock, 1888; Boschma, 1924; Yonge, 1936, 1953; Mansour, 1946a, b, c; Kawaguti, 1966; Fankboner, 1971; Goreau *et al.*, 1973). The only other recorded association of bivalves with dinoflagellates is the heart shell *Corculum cardissa*, which like the tridacnids is a member of the superfamily Cardiacea (Kawaguti, 1950, 1968).

Strong experimental evidence indicates that photosynthetic products of the algae are translocated to and used by the clams in a variety of anabolic and catabolic processes (Muscatine, 1967; Goreau *et al.*, 1973; Trench, 1979).

Morphologic and metabolic relations between the animals and the algae they harbor are unclear. According to Yonge (1936, 1953), the algae in the siphonal tissues, "invariably contained in blood amoebocytes," are periodically digested by the amoebocytes after transport to the viscera. The indigestible remains of the algae were hypothesized to accumulate in the enlarged kidneys (see Goreau *et al.*, 1973; Morton, 1978). However, other investigators have reported that the algae in the siphonal tissues are intercellular (Brock, 1888; Boschma, 1924; Mansour,

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Abbreviations: DCMU = (3[3,4 dichlorophenyl]-1,1 dimethyl urea, P_{max} = maximum net photosynthetic rate, K_m = radiant flux intensity at half-maximal photosynthetic rate, P_{gross} = gross photosynthesis (P_{net} + R), R = respiration, $P_{gross}/R = (P_{net} + R)/R$, PQ = photosynthetic quotient, RQ = respiratory quotient.

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1946a, b, c; Kawaguti, 1966; Bishop *et al.*, 1976). While Kawaguti (1966) and Fankboner (1971) agree that in the siphonal tissues the algae are intercellular, they suggest that in the viscera they are contained in and digested by amoebocytes (see also Morton, 1978). Fankboner (1971) presented electron microscopic histochemical evidence which he interpreted as demonstrating lysosomal digestion of the algae in the amoebocytes in the viscera. Yet Ricard and Salvat (1977) found live zooxanthellae in the feces of *T. maxima.*

On the question of the photosynthetic capabilities of the intact association, Yonge (1936) concluded that less oxygen was produced in the light by photosynthesis than was consumed in darkness, implying that the photosynthetic contribution of the algae was insufficient to offset the respiratory requirements of the consortium. In contrast, Wells *et al.* (1973) and Jaubert (1977) found net oxygen production by *Tridacna.*

Our studies resolve the conflicting information cited above, and indicate that:

(1) the algal symbionts in the siphonal tissues of *Tridacna maxima, T. crocea, T. squamosa, T. derasa, T. gigas,* and *Hippopus hippopus* are intercellular, and lie free in the haemal sinuses;

(2) the algae pass from the siphonal tissues to the gut, where they are voided in the feces;

(3) many such defecated symbionts are morphologically intact, photosynthetically active, and can be cultured;

(4) indigestible remains of symbiotic algae do not accumulate in the kidneys; indeed, the concretions found there are identified as calcium phosphate;

(5) the oxygen produced in daylight by *T. maxima*, even on cloudy days, exceeds that which is consumed at night, indicating net production by the association.

MATERIALS AND METHODS

Collection of specimens

Tridacnid clams were collected by diving off the reefs of the Republic of the Philippines; Enewetak Atoll, Marshall Islands; and Belau, Western Caroline Islands. Great care was taken when collecting to minimize damage and trauma, particularly with respect to the burrowing species. In the Philippines, animals were maintained shipboard on the *R. V. Alpha Helix*. Facilities at Enewetak were provided by the Mid Pacific Marine Laboratory, and in Belau by the Micronesia Mariculture Demonstration Center on Malakal. All animals were maintained in running sea water at ambient temperature, insolation, and salinity.

Histology

Whole small specimens were fixed intact in Bouin's or Carnoy's fixative for 24–48 h. The calcium carbonate remaining in the shell was removed by decalcification in repeated changes of 50% acetic acid. Specimens were dehydrated in methyl cellusolve (ethylene glycol monomethyl ether), cleared in xylene and dioxane, and embedded in Paraplast (Trench, 1974). Transverse serial sections 5–7 μm thick were mounted on glass slides and stained using Mallory tripple stain, haematoxylin and eosin, toluidine blue, haematoxylin and fast green FCF, Mallory phosphotungstic acid-haematoxylin, or the periodic acid-Schiff stain. The stained sections were examined and photographed with an Olympus Vanox microscope.

For ultrastructural studies, small pieces of tissues and fecal pellets were fixed

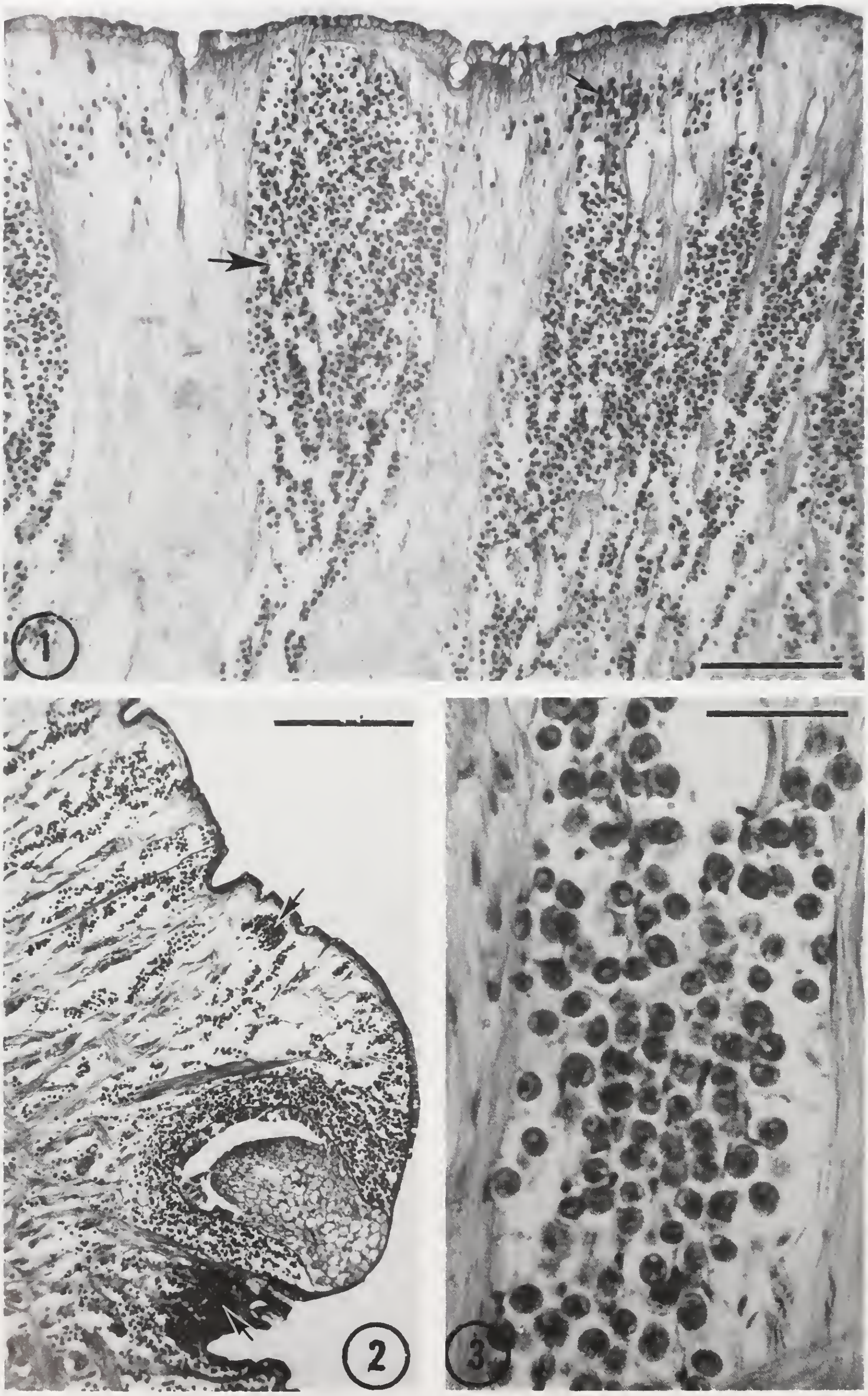

FIGURE 1. Light micrograph of a Paraplast-embedded section through the hypertrophied siphon of *T. gigas*. Heavy arrow indicates the haemal sinus containing the algae. Small arrow indicates the clusters of iridocytes. Scale bar = 200 μm.

in 6% cacodylate-buffered (pH 7.4) glutaraldehyde or in Karnovsky's fixative (Karnovsky, 1965). All fixations were conducted at 4°C. After washing in cacodylate buffer, samples were postfixed in cacodylate-buffered osmium tetraoxide (2%), dehydrated in serial changes of ethyl alcohol, and infiltrated and embedded in an Epon-Araldite mixture (Trench, 1974) or in Spurr's low viscosity embedding medium. This sections prepared on an LKB Ultratome V were viewed and photographed with a Siemens Elmiskop I.

Estimation of algal photosynthetic rates

Algae isolated from the siphonal tissues by the method of Trench (1971a) and those voided in feces were incubated in sea water containing added $NaH^{14}CO_3$ ($1.0\ \mu Ci \cdot ml^{-1}$), under approximately ambient conditions of temperature, and irradiance provided by cool white fluorescent lights. As controls, duplicate samples were incubated in the presence of DCMU (3[3,4 dichlorophenyl]-1,1 dimethyl urea) at a final concentration of $5 \times 10^{-6}\ M$. The DCMU, kindly supplied by E. I. DuPont de Nemours & Co., Wilmington, Delaware, was 2× recrystallized. After acidification and degassing to remove unincorporated inorganic ^{14}C, radioactive samples were assayed by liquid scintillation spectrometry. Appropriate corrections were made for quenching and background.

Chlorophyll was estimated after the algae were extracted in cold 90% acetone, and absorbancies measured at 664 and 630 nm. Calculations were based on the equations of Jeffrey and Humphrey (1975).

In situ *respirometry*

Oxygen fluxes were measured on *T. maxima* only. Two specimens, freshly collected from 5 m depth on the leeward side of Chinimi Island (Site Clyde), Enewetak Atoll, were used. Measurements were conducted *in situ* over 26 h periods (see McCloskey *et al.*, 1978) using polarographic electrodes and an automated recording system (Wethey and Porter, 1976a; Porter, 1980). Photosynthetic pigments were extracted in 90% acetone (20 h, dark, 5°C), extinctions were measured in 10 cm cells (Beckman Model DU Spectrophotometer), and chlorophyll *a* was estimated by the trichromatic equations of Richards (Strickland and Parsons, 1972).

Analysis of kidney concretions

Sections of kidney tissue mounted on glass slides were deparaffinized and coated with carbon. Analyses were conducted testing for Ca, Mg. Sr, P, As, and Sb, using an Electron Micro Probe Model EMX-5M120,000 (ARL, Sunland, Ca) at 15 KV. Standard apatite was used for comparison in the final analysis.

RESULTS

Morphological aspects of the symbiosis

Light microscopy showed that in the siphonal tissues of the six species of tridacnid clams, the symbiotic dinoflagellates are located within the haemal sinuses

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FIGURE 2. Light micrograph of a Paraplast-embedded section through the hypertrophied siphon of *T. gigas*, showing the "eye" and the associated algae. Arrows indicate clusters of iridocytes. Scale bar = 250 μm.

FIGURE 3. Light micrograph of a Paraplast-embedded section through the hypertrophied siphon of *T. crocea*, showing the algal symbionts in the haemal sinus. Scale bar = 30 μm.

(Fig. 1). Although in certain instances one may get the initial impression that the algae concentrate around the "lenses of the eyes" (see Stasek, 1966, and Fig. 2), observations on 3–10 specimens of each species showed that this is not the case. The relative density of zooxanthellae associated with the eyes, compared to other regions of the siphonal tissue, varies from sample to sample (*cf.* Yonge, 1936; Fankboner, 1981).

Light microscopy of Paraplast-embedded tissues and of 1-μm-thick Epon or Spurr-embedded tissues provided no evidence that the algae in the haemal sinuses were inside cells of any kind (Fig. 3). These observations were corroborated by ultrastructural observations (Fig. 4), which show the algae lying free in the haemal sinuses, but sometimes adhering to the sinus walls (*cf.* Goreau *et al.*, 1973). Algae isolated from the siphonal tissues by blending or mincing the tissue with a razor blade were never found in blood amoebocytes or any other cell when examined by phase contrast or Nomarski interference optics.

The symbiotic algae in tridacnids conform in their ultrastructural characteristics *in situ* (Fig. 4) to descriptions of *Symbiodinium* (= *Gymnodinium*) *microadriaticum* (Taylor, 1968, 1969, 1974; Kevin *et al.* 1969; Schoenberg and Trench, 1980a, b, c). Although there is confusion in the literature concerning the appropriate binomial for this organism (see Loeblich and Sherley, 1979; and Schoenberg and Trench, 1980b), we have selected the binomial suggested by Freudenthal (1962), based on arguments set forth in Trench (1981).

Within the haemal sinuses, we saw many algae undergoing binary fission. Surprisingly, we occasionally found obviously pycnotic algae (Fig. 5) in the haemal sinuses, but these algae were not contained within blood amoebocytes. The interpretation that pycnotic zooxanthellae indicate host digestion is unwarranted here (*cf.* Trench, 1974). Often, one or two pycnotic zooxanthellae were observed juxtaposed to morphologically intact zooxanthellae, all lying free in the haemal sinuses.

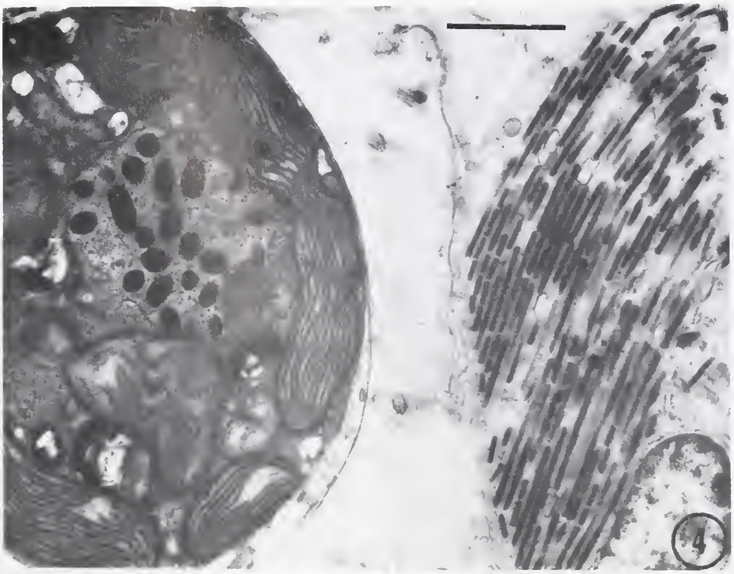
Movement of algal symbionts from siphonal tissues to rectum

Tridacnids collected and maintained in aquaria released brown fecal pellets within 24 h of collection. Light microscopy revealed many apparently intact zooxanthellae in these pellets, accompanied by unrecognizable debris and some material recognized as crustacean exoskeletal fragments. Clams maintained in aquaria continued to release fecal pellets with zooxanthelae for more than a month after collection.

To test the possibility that defecated algae were derived from pseudofeces, 15 clams were freshly collected, and 5 were fixed immediately in Bouin's fixative. For up to 2 weeks, five clams were maintained in aquaria with running unfiltered sea water and five in 0.22 μm Millipore-filtered sea water which was changed daily. Specimens were either fixed intact in Bouin's fixative; or dissected, and the stomach, digestive gland, intestine and rectum removed and prepared for light and electron microscopic analysis. Released fecal pellets were collected and treated in a similar manner.

Light microscopy of freshly collected intact clams revealed zooxanthellae in the lumina of the tubules of the digestive gland (Fig. 6), the stomach, the style sac, the intestine (Fig. 7), and the rectum. The algae appeared intact and many were dividing. Degraded pycnotic algae were also recognized, but zooxanthellae were never seen in the mantle chamber.

Examination of the feces released by freshly collected clams revealed that zooxanthellae and unrecognizable debris, probably mostly of plant origin (Fig. 8),



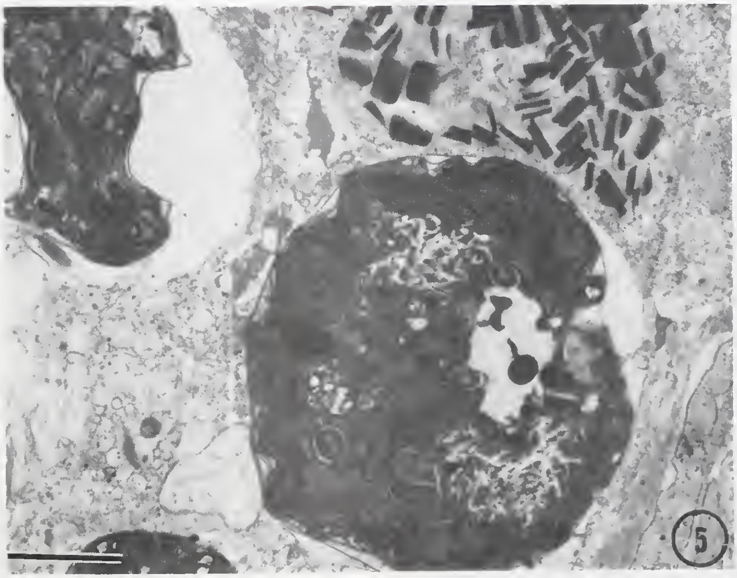
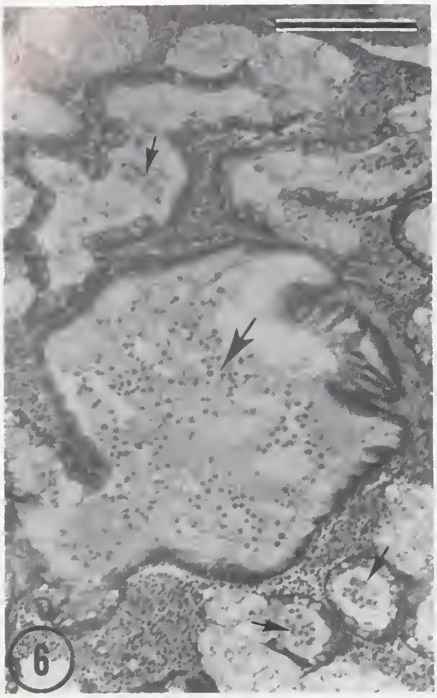
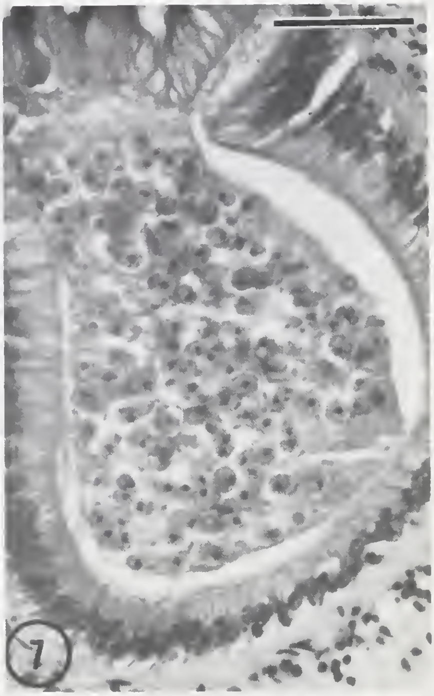


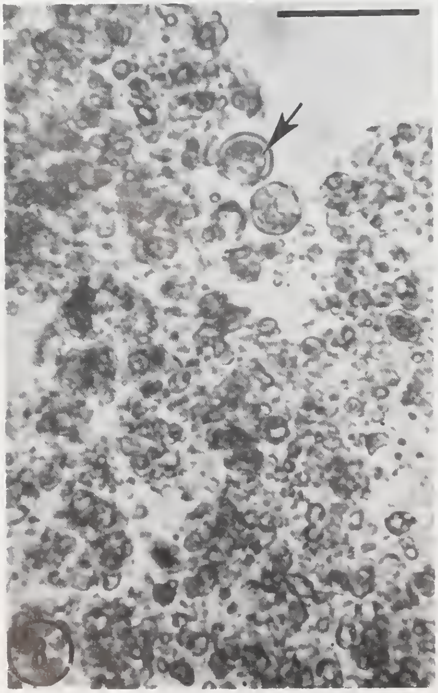
FIGURE 4. Transmission electron micrograph of *S. microadriaticum in situ* in the haemal sinus of *T. maxima*. The right hand side of the photograph shows an iridocyte which serves as a topological marker indicating siphonal tissue. Scale bar = 2 μm.

FIGURE 5. Transmission electron micrograph of two pycnotic *S. microadriaticum in situ* in the haemal sinus of *T. maxima*. An iridocyte is also shown. Scale bar = 2.5 μm.

were the major constituents. When animals were maintained in running sea water aquaria, the composition of the fecal pellets did not alter appreciably. However, when animals were maintained in filtered sea water, within 2–3 days the feces were composed almost exclusively of zooxanthellae (Fig. 9). Again, no zooxanthellae were seen in the mantle chamber. These observations suggest that the zooxanthellae







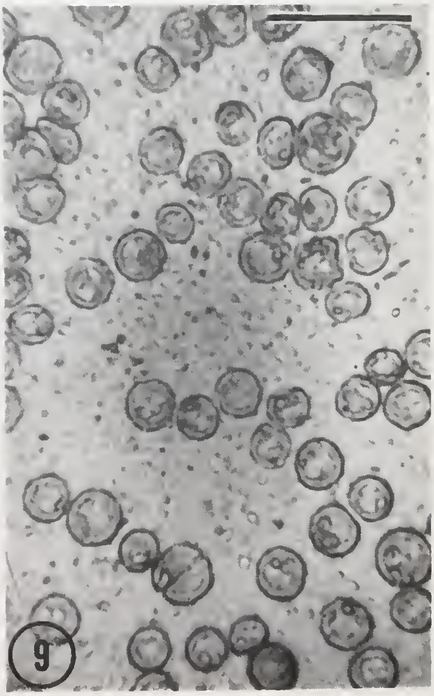


FIGURE 6. Light micrograph of a Paraplast-embedded section through the digestive diverticulum of *T. maxima*. Zooxanthellae can be seen in the lumina of the tubules of the digestive diverticulum (small arrows) and in the major collecting duct leading to the stomach (large arrow). Scale bar = 250 μm.

in the fecal pellets are not derived from pseudofeces and in addition indicate that the zooxanthellae in the feces are derived from the clam itself.

Electron microscopy (Fig. 10) confirmed the light microscopic observations, showing morphologically intact and pycnotic zooxanthellae, accompanied by unidentified debris and vesicles containing amorphous substances in the fecal pellets. Electron microscopic analysis of the contents of the rectum illustrated that many of the vesicles found in the fecal pellets were derived from macroapocrine secretions (Threadgold, 1976) originating in the epithelial cells lining the rectum (Fig. 11). Feces located in the lumen of the rectum contained intact zooxanthellae as well as zooxanthellae in various stages of disorganization (Fig. 12). None of these algae were found within cells. In addition to zooxanthellae, macroapocrine secretions, and debris, flagellated bacteria enclosed in vesicles (Fig. 13) were often observed.

Photosynthetic capacity of defecated symbionts

Comparisons of the photosynthetic rates of algae freshly isolated from the siphonal tissues, with those in the fecal pellets of freshly collected clams and clams maintained in Millipore-filtered sea water for 8 days, showed (Table I) that the rates of carbon fixation by the fecal pellets from *T. maxima* were somewhat less than, but comparable to, the rates of algae isolated from the siphonal tissues. However, DCMU inhibited carbon fixation in fecal pellets less than in freshly isolated algae, indicating that some carbon fixation in the fecal pellets was non-photosynthetic. The bacteria detected in the fecal pellets may be responsible for the non-photosynthetic CO_2 fixation (cf. Black *et al.*, 1976).

Similar patterns of separation of organic ^{14}C resulted from two dimensional radiochromatography (Trench, 1971a, b) of hot 80% ethyl alcohol extracts of labeled fecal pellets and algae isolated from the siphonal tissues (*cf.* Muscatine, 1973; Trench, 1971a, b). In addition, fecal pellets collected from *T. maxima, T. crocea, T. squamosa,* and *H. hippopus* produced viable cultures of *S. microadriaticum* when innoculated into the dinoflagellate growth medium ASP-8A (Ahles, 1967). These observations further support the conclusion that many of the symbiotic algae defecated by the clams are alive.

Identity of the kidney concretions

The kidneys of all six tridacnid species contained numerous concretions (Fig. 14) within cells of the tubules. The kidneys of juvenile *T. gigas* ($\leq$20 mm in length) contained a lower density of concretions than kidneys of larger adult clams, suggesting that the concretions may accumulate with age (*cf.* Yonge, 1936).

The concretions, about 12–25 μm in diameter, appear to be irregular to spherical concentric lamellations (Fig. 15). Electron microscopy (Fig. 16) confirmed the light microscope observations and showed that the concretions are not located in vacuoles.

To test whether the kidney concretions were related to indigestible remnants of zooxanthellae (Yonge, 1936; Morton, 1978), we analyzed extracts of the excised kidneys for photosynthetic pigments or degraded pigments (*e.g.* pheophytin or pheo-

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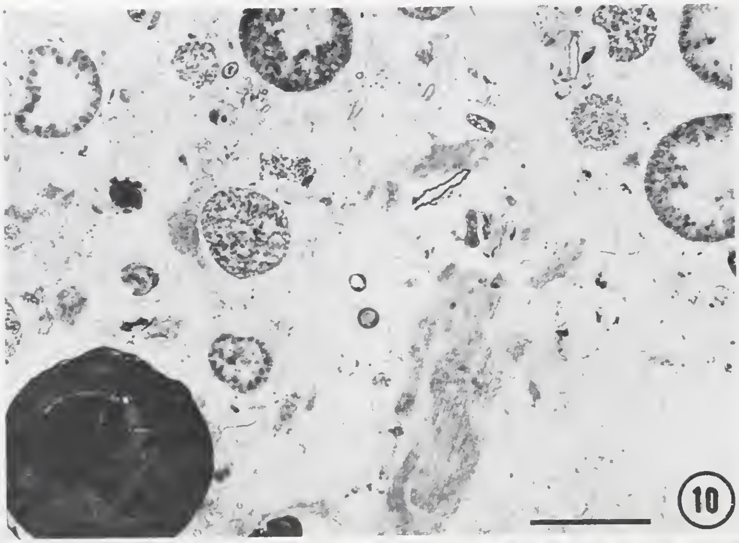
FIGURE 7. Light micrograph of a Paraplast-embedded section through the intestine of *T. crocea* at the point where it leaves the visceral mass but before entering the pericardium. Scale bar = 40 μm.

FIGURE 8. Light micrograph of a freshly collected fecal pellet from *T. gigas*,showing two intact zooxanthellae (arrow). The animal had been collected 4 h before the faecal pellet was discharged. Scale bar = 20 μm.

FIGURE 9. Light micrograph of a freshly collected fecal pellet from *T. gigas* maintained in Millipore-filtered seawater for 9 days. Scale bar = 20 μm.



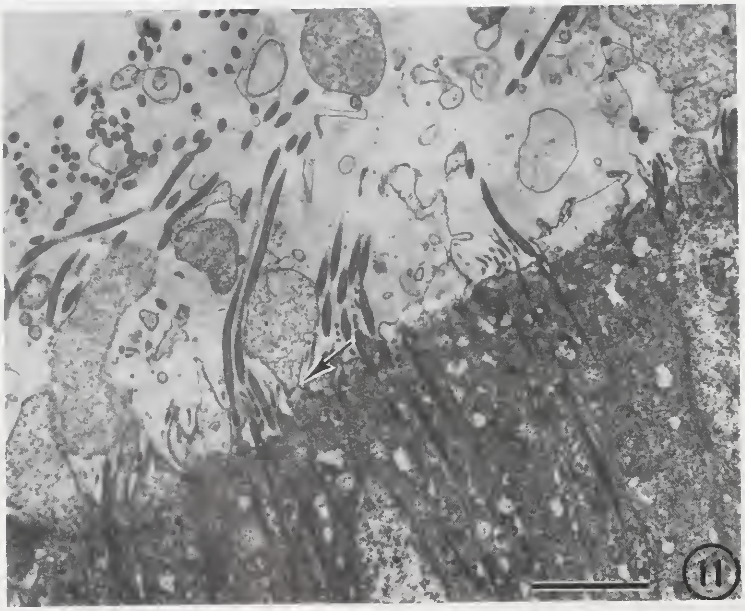
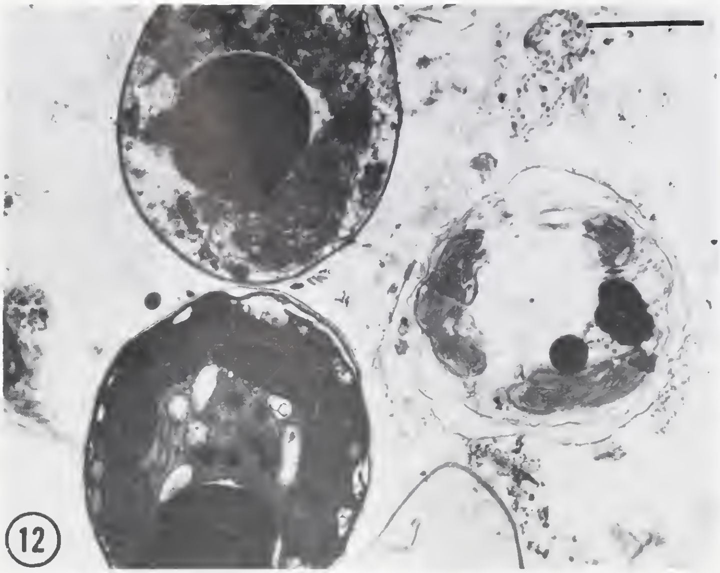


FIGURE 10. Transmission electron micrograph of a fecal pellet released by *T. derasa*. The one zooxanthella shown here appears to retain its morphological integrity. Note the many membrane-bound vesicles with amorphous contents. Scale bar = 4 μm.

FIGURE 11. Transmission electron micrograph of a section through the rectum of *T. derasa* showing the macroapocrine secretions in the lumen of the rectum and vesicles being formed but still attached to the epithelial cells (arrow). Scale bar = 5 μm.

phorbide), by spectrophotometry and by thin layer chromatography. No pheophytin or pheophorbide were detected. Minute traces of chlorophyll and peridinin were detected (*cf.* Ricard and Salvat, 1977). These probably came from the zooxanthellae often seen in vessels (probably blood vessels) that passed through the kidneys (Fig.



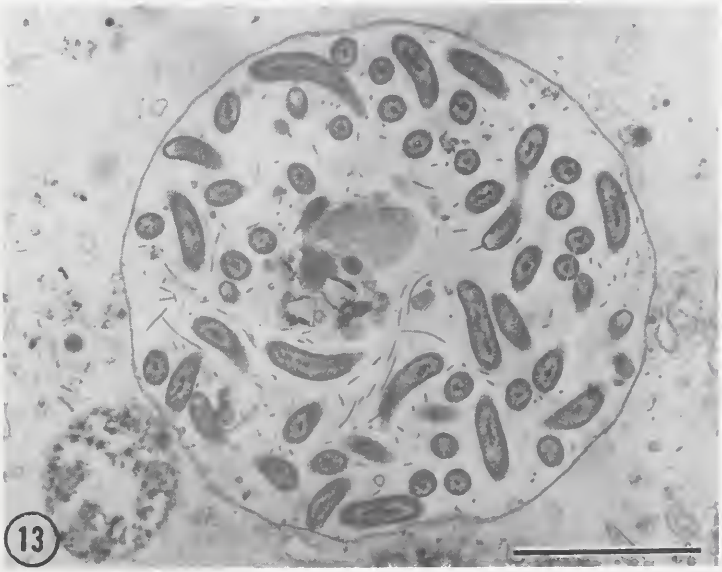


FIGURE 12. Transmission electron micrograph of a section through a fecal pellet in the rectum of *T. derasa* showing one morphologically intact zooxanthella and two pycnotic algal cells. Scale bar = 2.8 μm.

FIGURE 13. Transmission electron micrograph of a section through the rectum of *T. squamosa* showing the flagellated bacteria in a vesicle in the fecal pellet. Scale bar = 2 μm.

14), and sometimes seen also in connective tissue covering the kidneys, though never in the cells of the kidneys themselves.

Finally, electron microprobe analysis of the kidney concretions indicated the presence of calcium and phosphate in quantities similar to that found in apatite. No magnesium, strontium, arsenic, or antimony were detected. We therefore con-

TABLE I

Rates of photosynthetic CO_2 fixation by S. microadriaticum *from* T. maxima *at 25°C and 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$. By comparison with Fig. 17, this radiant flux was about 50% saturation.)*

	*mg $C \cdot (mgChl_a)^{-1} \cdot h^{-1}$	% Inhibition by DCMU ($5 \times 10^{-6} M$)
Freshly isolated algae from siphonal tissues	3.6–4.8	98–99
Fecal pellets from freshly collected animals	0.4–2.1	80–87
Fecal pellets from animals maintained in filtered seawater for 8 days	1.9–4.0	90–95

* Numbers represent range of values based on four assays involving four different specimens.

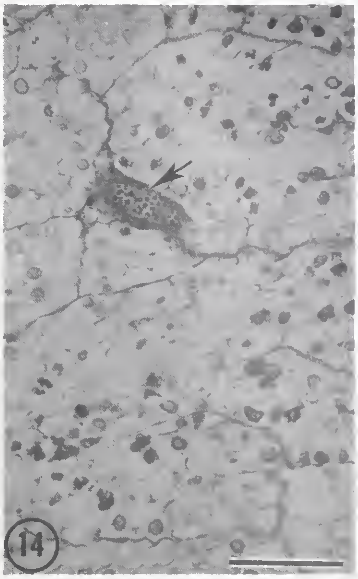
clude that the kidney concretions are biogenic phosphorite, probably homologous to phosphorite concretions found in the kidneys of other bivalve molluscs (Doyle *et al.* 1978), which do not harbor symbiotic algae.

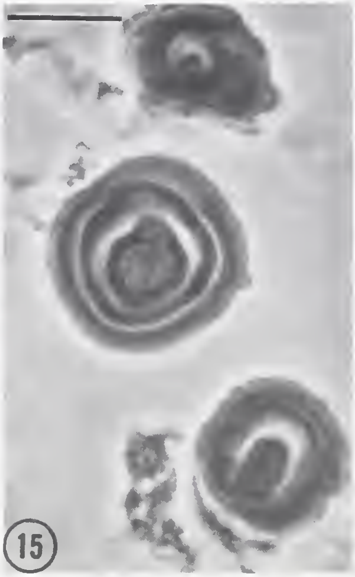
Oxygen production and consumption by T. maxima

Two clams were used to study oxygen fluxes. The first clam had a maximum shell length of 84.8 mm, a maximum shell width of 38.5 mm, and an internal shell volume of 60 ml. The dry weight of the tissue was 4.24 g and the nitrogen content was 9.9% dry weight. The algae in the clam contained 0.38 mg chlorophyll *a*. The second clam had a maximum shell length of 87.4 mm, a maximum shell width of 45.5 mm, and an internal shell volume of 66 ml. The dry weight of the tissue was 4.85 g and the nitrogen content was 9.5% dry weight. Chlorophyll *a* content was 0.56 mg.

Figure 17 shows the net photosynthetic oxygen production of the two clams in relation to radiant flux intensity (within the photosynthetically active wavelength range 400–700 nm). The data were fitted to the Michaelis-Menten equation (Wethey and Porter 1976a; McCloskey *et al.*, 1978). Based on regression analyses of idealized curves of the photosynthesis-radiant flux relation (Bliss and James, 1966; Wethey and Porter, 1976a, b) we found an average maximum net photosynthesis (P_{max}) of 46.0 (range, 30–57) mg $0_2 \cdot$ mg Chl $a^{-1} \cdot h^{-1}$. One clam produced 329.0 mg$O_2 \cdot$ mg Chl a^{-1} during 12 h of daylight, and consumed 41.0 mg O_2 during 12 h of darkness, while the second clam produced 342.8 mg $O_2 \cdot$ mg Chl a^{-1} and consumed 70.0 mg O_2 during the same periods. The radiant flux intensity at half-maximal photosynthetic rate (K_m) is a measure of the affinity of the photosynthetic system for light. Our estimates of K_m for these two individuals of *T. maxima* were 1.61 (95% confidence limits 1.05–2.40) and 0.94 (95% conf. lim. 0.49–1.57) Einsteins (E)$\cdot m^{-2} \cdot h^{-1}$, respectively.

To model gross photosynthesis and respiration over 24 h, we used the regression equations for the photosynthesis-radiant flux relations (McCloskey *et al.*, 1978). We recorded radiant flux with a quantum sensor on shore for 3 weeks at Enewetak and *in situ* during the experiments. We calculated the expected radiant flux at depth for theoretical sunny (maximum radiant flux 8.64 E $\cdot m^{-2} \cdot h^{-1}$) and cloudy (maximum radiant flux 4.32 E$\cdot m^{-2} \cdot h^{-1}$) days using surface recordings and measured light extinction coefficients. The theoretical daily time courses of radiant flux were divided into 1 h increments and the expected production of oxygen was cal-





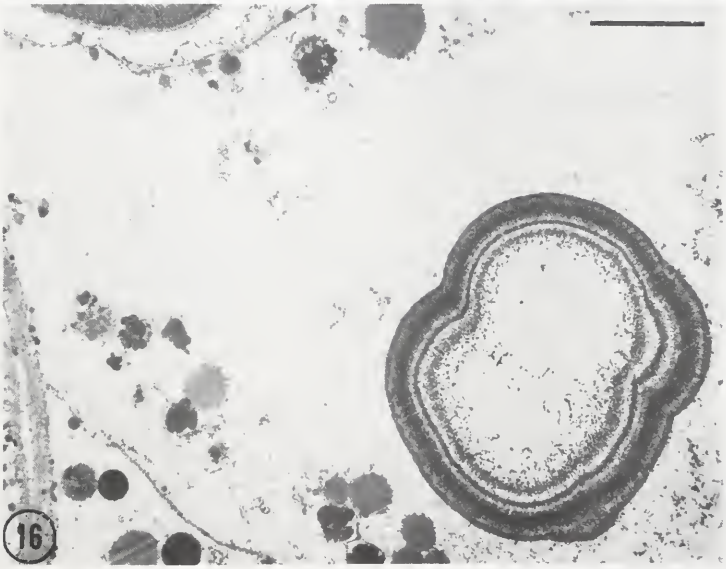


FIGURE 14. Light micrograph of a Paraplast-embedded section through the kidney of *T. crocea* showing the distribution of the concretions. The arrow indicates the "blood vessel" containing zooxanthellae. Scale bar = 170 μm.

FIGURE 15. Light micrograph of kidney concretions in *T. crocea* stained with toluidine blue. Scale bar = 15 μm.

FIGURE 16. Transmission electron micrograph of a portion of *T. gigas* kidney, showing details of the kidney cell and the phosphorite concretion. Scale bar = 9 μm.

culated for each increment using the regression equations of the photosynthesis-radiant flux relations (Wethey and Porter, 1976b). The ratio of gross oxygen production to consumption (P_{gross}/R) over 24 h was estimated as 2.02 (95% conf. lim.

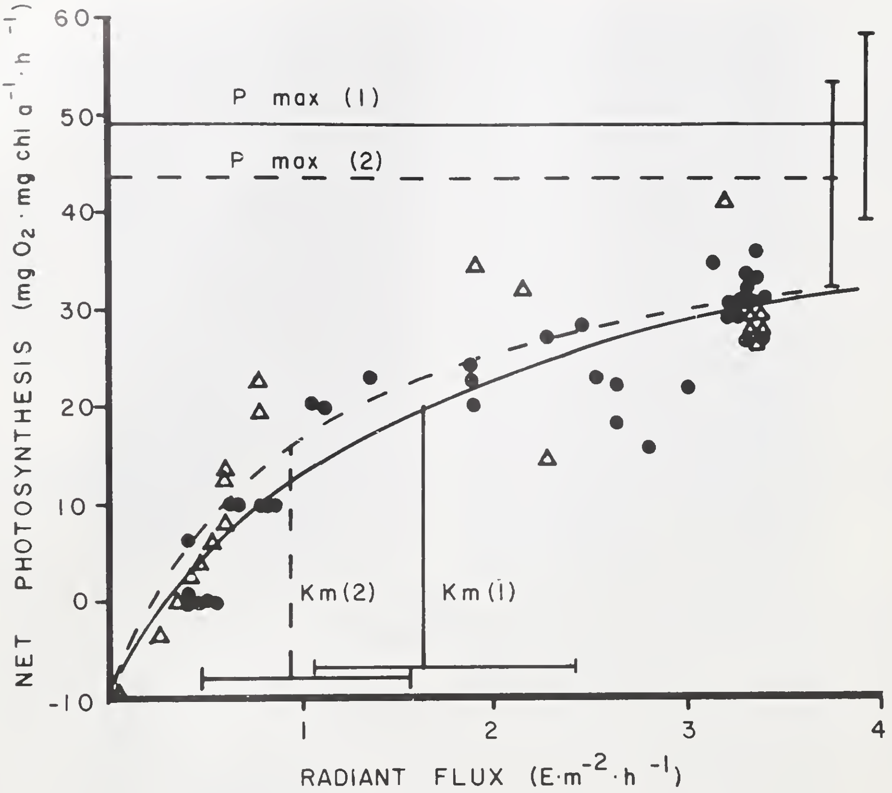


FIGURE 17. The net photosynthesis-radiant flux relation for two speciments of *Tridacna maxima* (solid circle and open triangle respectively) collected and assayed at 5 m depth on Enewetak Atoll. Fitted curves, maximum net photosynthetic rates (P_{max}), radiant flux intensities at half saturation (K_m), and their 95% confidence limits are from regression analyses of Bliss and James (1966).

1.67–2.37) and 1.86 (95% conf. lim. 1.51–2.21) for the two specimens on a theoretical sunny day, and 1.52 (95% conf. lim. 1.26–1.78) and 1.50 (95% conf. lim. 1.22–1.79) on a theoretical cloudy day.

Net oxygen production and a $P_{gross}/R > 1.0$ in plant-animal symbioses only become significant if photosynthetically fixed carbon is transported from the algae to the animals, and the animals use such translocated carbon. Muscatine (1967) demonstrated that algae from *T. crocea* released photosynthate *in vitro.* Goreau *et al.* (1973) showed the same phenomenon *in vivo* in *T. maxima*, and in addition illustrated that the translocated carbon was used in animal metabolism.

To estimate the relative contribution of algal photosynthesis to the respiratory carbon demand of the animals, we used the model of Porter (1980). In this model, the percent contribution of zooxanthellar carbon to animal daily requirements for respiration (%C) is related to the net carbon assimilated by zooxanthallae ($P_{net}z$), the percent of net carbon subsequently translocated from the zooxanthellae to the clam (% Tr), and the carbon respired by the animal (R anim), by the following equation:

$$\%\,C = \frac{[P_{net}z\ (24\ h)](\%Tr)}{R\ anim\ (24\ h)} \tag{1}$$

However, oxygen flux data do not directly provide values for any of these terms. What we measure in the *in situ* respirometer is net oxygen production by the algae and the clam in daylight (P_{net} clam, daylight), and whole clam respiration at night (R clam, night). Since clam respiration is the sum of animal and algal respiration (R clam = R zoox + R anim), and since net clam production is the difference between gross algal photosynthesis and the sum of algal and animal respiration [P_{net} clam = P_{gross} zoox − (R zoox + R anim)], we need to make some assumptions to estimate the values of three variables in order to express the above theoretically accurate equation in an empirically determinable form. We assumed that a clam's daytime and nighttime respiration were equal, and that P_{net} zoox (daytime) = P_{net} zoox (24 h). The logic for these assumptions is discussed in Porter (1980) and Muscatine (1980). These assumptions allow us to calculate P_{net} zoox (24 h) and R anim (24 h) from measurements of P_{net} clam and R clam (night). We assumed an algal biomass and algal contribution to intact clam respiration of 5% (see McCloskey *et al.*, 1978; Muscatine and Porter, 1977; Muscatine, 1980), although it could conceivably vary between 1–10%. We arbitrarily gave a value of 1.0 to the photosynthetic quotient (PQ) and the respiratory quotient (RQ). Finally, using the ^{14}C technique, the measured release of photosynthetically fixed carbon by the algae from *T. maxima* was 39–45% (Trench, unpublished). We set this variable at 40%. The oxygen data were converted to carbon equivalents (McCloskey *et al.*, 1978).

Using the above assumptions and the described parameter estimates, Equation 1 can be expressed in terms of our measurable variables:

$$\%C = \frac{\begin{array}{c}[(P_{net}\text{clam, 12 h day})(.375\ PQ^{-1}) \\ + (\text{R clam, 12 h night})(.375\ RQ)(95\%)](40\%)\end{array}}{(\text{R clam, 12 h night})(.375\ RQ)(2)(95\%)} \cdot 100 \qquad (2)$$

The model indicates that on a theoretical sunny day the algae may contribute 84% (95% conf. lim. 65–93%) and 77% (95% conf. lim. 63–92%), respectively, of the respiratory carbon needed by the animals. On a theoretical cloudy day they may contribute 63% (95% conf. lim. 53–75%) and 62% (95% conf. lim. 51–74%). Thus, the photosynthetic products of the algae may provide more than half the respiratory carbon requirements of the clams even on overcast days. Our measurements were made in October; in mid-summer, the contribution of the algae could be higher.

Discussion

The classic paper on the biology of symbiosis with zooxanthellae among tridacnid bivalves (Yonge, 1936) suggests, and subsequent papers (Yonge, 1953; Goreau *et al.*, 1973) reiterate, that the symbiotic algae in the tissues of the hypertrophied siphon occupy the haemal sinuses, and are "invariably contained within blood amoebocytes." These "farmed" algae were believed to be digested intracellularly by the amoebocytes in the viscera, and their indigestible remains to accumulate as concretions in the enlarged kidneys (see also Morton, 1978). Our data are inconsistent with these conclusions.

Light and electron microscopic analyses of the algae *in situ* unambiguously showed zooxanthellae in the haemal sinuses of the siphonal tissues, but no evidence of their being in amoebocytes or any other cell type was found. This is in contrast to the situation observed in several coelenterates (Trench, 1979, 1980, 1981; Trench *et al.*, in press). Our conclusion that the algae in the siphonal tissues of tridacnids are intercellular agrees with the early reports of Brock (1888), Boschma (1924), and Mansour (1946a, b, c), and with recent observations by Kawaguti (1966), Fankboner (1971), and Bishop *et al.* (1976).

Since the algal symbionts are in blood sinuses, they might be expected in other areas of the clam through which blood circulates. Indeed, Yonge (1936) observed zooxanthellae in the gills and the heart tissue, but Mansour (1946b) could not corroborate his observations. We saw zooxanthellae in the ventricle and in a vessel passing through the kidney (Fig. 14). This vessel could be synonymous with Mansour's tubular system connecting the siphon to the stomach, which Yonge (1953) discounted. We have not been able to identify Mansour's system of tubes either. The important point is that even when zooxanthellae are found in tissues other than the siphon (with the exception of the digestive gland), they are not within amoebocytes. The situation with respect to the digestive diverticulum will be dealt with elsewhere.

Obviously pycnotic zooxanthellae in the siphonal tissues (Fig. 5), often juxtaposed to morphologically intact algae, raise questions about the significance of moribund algae in tridacnids. Traditionally, pycnotic algae have been interpreted as evidence of animal digestion, usually occurring intracellularly, effected by the enzymes contained within lysosomes. However, pycnotic algae lying free in the haemal sinuses of the siphonal tissues are inconsistent with this interpretation. These algae are reminiscent of the situation in *Zoanthus sociatus*, where Trench (1974) suggested that the pycnotic algae were a result of autolytic degradation (Trench *et al.*, 1981).

In his early reports, Mansour (1946b) described intact zooxanthellae and zooxanthellae in various stages of degradation in the alimentary tract of *T. elongata* (= *maxima*). Yonge (1936) had also observed these, but concluded that they were artifactual, resulting from damage to the clams during handling.

Our study of the passage of algae from the siphonal tissues to the alimentary tract was initiated because of its similarity to the release of pellets containing pycnotic algal symbionts by zoanthids (Reimer, 1971; Trench, 1974) and live algae by sea anemones (Steele, 1975, 1977).

Since the clams continued to release viable zooxanthellae even after extended maintenance in filtered sea water, the defecated algae cannot be derived from the surrounding environment, but must be derived from the clam itself (*cf.* Fankboner and Reid, 1981). Histological sections of fixed intact clams showed no evidence of zooxanthellae in the mantle chamber, eliminating the possibility that the pellets are pseudofeces. Many zooxanthellae in the lumina of the digestive diverticulum, the stomach, the intestine, and the rectum (none of which were inside cells) were morphologically intact, but moribund cells in various stages of degradation were also observed. As the defecated algae were photosynthetically functional and could be cultured, they must have passed from the siphonal tissues through the digestive tract without being digested.

We do not know the mechanism by which the algae pass from the haemal spaces to the alimentary tract. Morton (1978) has proposed that digested zooxanthellae are discharged from the amoebocytes in the visceral mass to the lumina of the diverticulum when the epithelial lining of the tubules "break down" in a cyclical diurnal manner. We cannot deny or corroborate this, but the digestive diverticulum seems to be the most probable site where the algae enter the alimentary tract.

The evidence supporting the concept that tridacnids digest their symbionts is often inconclusive. Fankboner (1971) used the electron microscopic histochemical visualization of acid phosphatase in support of digestion of the algae in amoebocytes. However, this method does not resolve the source of the enzyme, merely the existence of enzyme activity. Trench *et al.* (1981) showed that *S. microadriaticum* possessed several isoenzymes of acid phosphatase. Hence, demonstrating acid phosphatase associated with algae in electron micrographs does not resolve the source of the enzyme. Our observations raise further doubt that tridacnids digest their symbiotic algae. Several independent studies indicate that bivalves are not able to digest and assimilate algal material from all sources uniformly (Coe, 1948; Dean, 1958; Haven and Morales-Alamo, 1966; and Hildreth, 1980). In addition, studies on the process of infection of juvenile *Tridacna squamosa* by *S. microadriaticum* (Fitt and Trench, in preparation) indicate that the algae enter the animals via the feeding apparatus and are somehow passed undigested from the alimentary tract to the siphon, wherein they proliferate.

The function of the macroapocrine secretions in the rectum of tridacnids is not known, but since it results in loss of cell membranes, our observations suggest that membrane turnover in the rectum of *Tridacna* may be very high. This particular mode of secretion has previously been associated with glandular tissues such as the submandibular gland of rabbits and the mammary gland (Threadgold, 1976). Although not previously reported in rectal tissue, a similar phenomenon has recently been reported in the jejunal cells of hamsters (Misch *et al.*, 1980).

The enlarged kidney of tridacnids has been regarded as the repository for the indigestible remains of zooxanthellae (Yonge, 1936; Goreau *et al.*, 1973; Morton, 1978). Our evidence does not support this conclusion. No degraded zooxanthellar pigments were identified in kidney extracts and the small quantities of chlorophyll and peridinin detected were readily traced to zooxanthellar contamination in tissues associated with the kidney. The kidney concretions are in fact phosphorite, and are common in bivalves, for example *Mercenaria* and *Argopecten* (Doyle *et al.*, 1978), which do not harbor symbiotic algae.

The existence of phosphorite concretions in the kidney of tridacnid clams raises questions on their source and mechanism of formation. It seems to us somewhat paradoxical that organisms living in phosphate-depleted environments such as coral reefs should "deposit" such large quantities of this mineral. Perhaps views on phosphate depletion in coral reef environments should be reassessed. However, *if* the phosphate is not irreversibly "locked up" in the concretions, then they could potentially serve as a reservoir of phosphorus. Obviously, such speculation needs to be experimentally tested.

The relation between net photosynthesis and radiant flux indicates that the algae in the clams living at 5 m are adapted to strong light, compared to corals such as *Pavona praetorta, Plerogyra sinuosa,* and *Porites lutea* from 10–24 m depth. The estimated K_m values for *T. maxima* were 0.94 and 1.61 $E \cdot m^{-2} \cdot h^{-1}$ as compared to values ranging from 0.11 to 0.65 $E \cdot m^{-2} \cdot h^{-1}$ for the corals (Wethey and Porter, 1976a, b). Net photosynthesis in *T. maxima* shows the same noonday peak as the corals (Porter, 1980) and shows no sign of photoinhibition even at the highest radiant flux intensities (Fig. 17).

Based on regression analyses of idealized curves of the production-radiant flux relation (Bliss and James, 1966; Wethey and Porter, 1976a, b), the P_{max} values in *T. maxima* are about five times higher than in *P. praetorta*, but the P_{gross}/R values for the two organisms are very similar, reflecting the higher animal biomass and respiration in *T. maxima.*

Since the algae produce oxygen at a high rate in the haemal sinuses of *T. maxima*, and since high levels of oxygen are potentially toxic or could cause embolisms, how is the excess oxygen removed from the system? Diffusion across the mantle surface is one possibility. Transport via the blood to the gills, where oxygen is unloaded and eliminated via the excurrent siphon, is an alternative, but this would be the reverse of the normal process of oxygen loading and unloading in bivalves generally. Measuring oxygen tension at the mantle surface and in the afferent and efferent blood vessels associated with the gills during high photosynthesis should resolve this.

Our calculated estimates of the relative contribution of zooxanthellar photosynthesis to the respiratory carbon demands of the animals suggest that the algae represent a major source of metabolic carbon. This conclusion is provisionally supported by the studies of Muscatine (1967) and Goreau *et al.* (1973). However, the percent contribution values should not be overemphasized, because of the many different assumptions that must be made in any attempt to quantify the role of the algae in the symbiosis (Muscatine, 1980).

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LITERATURE CITED

AHLES, M. D. 1967. Some aspects of the morphology and physiology of *Symbiodinium microadriaticum*. Ph.D. dissertation, Fordham Univ., N. Y. 158 pp.

BISHOP, D. G., J. M. BAIN, AND W. J. S. DOWNTON. 1976. Ultrastructure and lipid composition of zooxanthellae from *Tridacna maxima*. *Aust. J. Plant Physiol.* **3:** 33–40.

BLACK, C. B., JR., J. E. BURRIS, AND R. G. EVERSON. 1976. Influence of oxygen concentration on photosynthesis in marine plants. *Aust. J. Plant Physiol.* **3:** 81–86.

BLISS, C. I., AND A. T. JAMES. 1966. Fitting the rectangular hyperbola. *Biometrics* **22:** 573–602.

BOSCHMA, H. 1924. On the food of Madreporaria. *Proc. Acad. Sci. Amsterdam.* **27:** 13–23.

BROCK, J. 1888. Über die sogenannten Augen von *Tridacna* und das vorkommen von Pseudochlorophyllkörpen im Gëfass system der Muscheln. *Z. Wiss. Zool.* **66:** 270–288.

COE, W. R. 1948. Nutrition, environmental conditions and growth of marine bivalve mollusks. *J. Mar. Res.* **7:** 586–601.

DEAN, D. 1958. New property of the crystalline style of *Crassostrea virginica*. *Science* **128:** 837.

DOYLE, L. J., N. J. BLAKE, C. C. WOO, AND P. YEVICH. 1978. Recent biogenic phosphorite: Concretions in mollusk kidneys. *Science* **199:** 1431–1433.

FANKBONER, P. V. 1971. Intracellular digestion of symbiotic zooxanthellae by host amoebocytes in giant clams (Bivalvia: Tridacnidae), with a note on the nutritional role of the hypertrophied siphonal epidermis. *Bio. Bull.* **141:** 222–234.

FANKBONER, P. V. 1981. Siphonal eyes of giant clams (Bivalvia, Tridacnidae) and their relationship to adjacent zooxanthellae. *Veliger* **23:** 245–249.

FANKBONER, P. V., AND R. G. REID. 1981. Mass expulsion of zooxanthellae by heat-stressed reef corals: a source of food for giant clams? *Experientia* **37:** 251–252.

FREUDENTHAL, H. D. 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: Taxonomy, life cycle and morphology. *J. Protozool.* **9:** 45–52.

GOREAU, T. F., N. I. GOREAU, AND C. M. YONGE. 1973. On the utilization of photosynthetic products from zooxanthellae and of a dissolved amino acid in *Tridacna maxima* f. *elongata* (Mollusca: Bivalvia). *J. Zool. Lond.* **169:** 417–454.

HAVEN, D. S., AND R. MORALES-ALAMO. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limnol. Oceanogr.* **11:** 487–498.

HILDRETH, D. L. 1980. The passage of two species of micro-algae through the gut of *Mytilus edulis* (L.) *J. Exp. Mar. Biol. Ecol.* **48:** 17–22.

JAUBERT, J. 1977. Light, metabolism and the distribution of *Tridacna maxima* in a South Pacific atoll: Takapoto (French Polynesia). Pp. 489–494 in D. L. Taylor, Ed., *Proceedings Third International Coral Reef Symposium.* University of Miami Press, Coral Gables.

JEFFREY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen.* **167:** 191–194.

KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27:** 137A.

KAWAGUTI, S. 1950. Observations on the heart shell, *Corculum cardissa* (L.) and its associated zooxanthellae. *Pacific Sci.* **4:** 43–49.

KAWAGUTI, S. 1966. Electron microscopy on the mantle of the giant clam with special reference to zooxanthellae and iridophores. *Biol. J. Okayama Univ.* **12:** 81–92.

KAWAGUTI, S. 1968. Electron microscopy on zooxanthellae in the mantle and gill of the heart shell. *Biol. J. Okayama Univ.* **14:** 1–12.

KEVIN, M., W. T. HALL, J. J. A. MCLAUGHLIN, AND P. A. ZAHL. 1969. *Symbiodinium microadriaticum* Freudenthal, a revised taxonomic description. *J. Phycol.* **5:** 341–350.

LOEBLICH, A. R., AND J. L. SHERLEY. 1979. Observations on the theca of the motile phase of free living and symbiotic isolates of *Zooxanthella microadriatica* (Freudenthal) comb. nov. *J. Mar. Biol. Assoc. U. K.* **59:** 195–205.

MANSOUR, K. 1946a. Communication between the dorsal edge of the mantle and the stomach of *Tridacna. Nature* **157:** 844.

MANSOUR, K. 1946b. Source and fate of the zooxanthellae of the visceral mass of *Tridacna elongata. Nature* **158:** 130.

MANSOUR, K. 1946c. The zooxanthellae, morphological peculiarities and food and feeding habits of the Tridacnidae with reference to other lamellibranches. *Proc. Egypt Acad. Sci.* **1:** 1–11.

MCCLOSKEY, L. R., D. S. WETHEY, AND J. W. PORTER. 1978. Measurement and interpretation of photosynthesis and respiration in reef corals. *Monogr. Oceanogr. Methodol. (UNESCO)* **5:** 379–396.

MISCH, D. W., P. E. GIEBEL, AND R. G. FAUST. 1980. Intestinal microvilli: responses to feeding and fasting. *Eur. J. Cell Biol.* **21:** 269–279.

MORTON, B. 1978. The diurnal rhythm and the process of feeding and digestion in *Tridacna crocea* (Bivalvia: Tridacnidae). *J. Zool. (Lond.)* **185:** 371–387.

MUSCATINE, L. 1967. Glycerol excretion by symbiotic algae from corals and *Tridacna*, and its control by the host. *Science* **156:** 516–519.

MUSCATINE, L. 1973. Nutrition of corals. Pp. 77–115 in O. A. Jones and R. Endean, Eds. *Biology and Geology of Coral reefs.* Academic Press, New York and London.

MUSCATINE, L. 1980. Productivity of zooxanthellae. *Brookhaven Symp.* **31:** 381–402.

MUSCATINE, L. AND J. W. PORTER. 1977. Reef corals: Mutualistic symbioses adapted to nutrient poor environments. *Bio Science* **27:** 454–460.

PORTER, J. W. 1980. Primary productivity in the sea: reef corals *in situ. Brookhaven Symp.* **31:** 403–410.

REIMER, A. A. 1971. Feeding behavior in Hawaiian zoanthids *Palythoa* and *Zoanthus. Pacific Sci.* **25:** 512–520.

RICARD, M., AND B. SALVAT. 1977. Faeces of *Tridacna maxima* (Mollusca: Bivalvia); composition and coral reef importance. Pp. 495–501 in D. L. Taylor, Ed., *Proceedings Third International Coral Reef Symposium.* University of Miami, Coral Gables, Fl.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980a. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *Microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. I. Isoenzyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum. Proc. R. Soc. Lond. B. Biol. Sci.* **207:** 405–427.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980b. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. II. Morphological variation in *Symbiodinium microadriaticum. Proc. R. Soc. Lond. B. Biol. Sci.* **207:** 429–444.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980c. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. III. Specificity and infectivity of *Symbiodinium microadriaticum. Proc. R. Soc. Lond. B. Biol. Sci.* **207:** 445–460.

STASEK, C. F. 1966. The eye of the giant clam (*Tridacna maxima*). *Occ. Pap. Calif. Acad. Sci.* **58:** 1–9.

STEELE, R. D. 1975. Stages in the life history of a symbiotic zooxanthella in pellets extruded by its host *Aiptasia tagetes* (Duch. and Mich.) (Coelanterata, Anthozoa). *Biol. Bull.* **149:** 590–600.

STEELE, R. D. 1977. The significance of zooxanthella containing pellets extruded by sea anemones. *Bull. Mar. Sci.* **27:** 591–594.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A Practical Handbook of Seawater Analysis. *Bull. Fish. Res. Bd. Canada.* **167:** 1–310.

TAYLOR, D. L. 1968. *In situ* studies on the cytochemistry and ultrastructure of a symbiotic marine dinoflagellate. *J. Mar. Biol. Assoc. U. K.* **48:** 349–366.

TAYLOR, D. L. 1969. Identity of zooxanthellae isolated from Pacific Tridacnidae. *J. Phycol.* **5:** 336–340.

TAYLOR, D. L. 1974. Symbiotic marine algae: taxonomy and biological fitness. Pp. 245–262 in W. B. Vernberg, Ed. *Symbiosis in the Sea.* University of South Carolina Press, Columbia, SC.

THREADGOLD, L. T. 1976. *The Ultrastructure of the Animal Cell.* Pergamon Press. 514 pp.

TRENCH, R. K. 1971a. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. II. Liberation of fixed ^{14}C by zooxanthellae *in vitro. Proc. R. Soc. Lond. B. Biol. Sci.* **177:** 237–250.

TRENCH, R. K. 1971b. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. III. The effects of homogenates of host tissues on the excretion of photosynthetic products *in vitro* by zooxanthellae from two marine coelenterates. *Proc. R. Soc. Lond. B. Biol. Sci.* **177:** 251–264.

TRENCH, R. K. 1974. Nutritional potentials in *Zoanthus sociatus* (Coelenterata: *Anthozoa*). *Helgolander. Wiss. Meeresunters.* **26:** 174–216.

TRENCH, R. K. 1979. The cell biology of plant-animal symbiosis. *Ann. Rev. Plant Physiol.* **30:** 485–531.

TRENCH, R. K. 1980. Integrative mechanisms in mutualistic symbioses. Pp. 275–297 in C. B. Cook, P. W. Pappas and E. D. Rudolph, Eds. *Cellular Interactions in Symbiosis and Parasitism.* Ohio State Univ. Press, Columbus.

TRENCH, R. K. 1981. Cellular and molecular interactions in symbioses between dinoflagellates and marine invertebrates. *Pure App. Chem.* **53:** 819–835.

TRENCH, R. K., N. J. COLLEY, AND W. K. FITT. 1981. Recognition phenomena in symbioses between marine invertebrates and "zooxanthellae"; uptake, sequestration and persistence. *Ber. Deutsch Bot. Ges.* **94:** 568–577.

WELLS, J. M., A. H. WELLS, AND J. G. VANDERWALKER. 1973. *In situ* studies of metabolism in benthic reef communities. *Helgolander. Wiss. Meeresunters.* **24:** 78–81.

WETHEY, D. A., AND J. W. PORTER. 1976a. Sun and shade differences in productivity of reef corals. *Nature* **262:** 281–282.

WETHEY, D. A., AND J. W. PORTER. 1976b. Habitat-related patterns of productivity of the foliaceous reef-coral, *Pavona praetorta.* Pp. 59–66 in G. O. Mackie, Ed. *Coelenterate Ecology and Behaviour.* Plenum Press, New York.

YONGE, C. M. 1936. Mode of life, feeding, digestion and symbiosis with zooxanthellae in the Tridacnidae. *Sci. Rep. Gr. Barrier Reef Exped. Brit. Mus. (Nat. Hist.)* **1:** 283–321.

YONGE, C. M. 1953. Mantle chambers and water circulation in the Tridacnidae. *Proc. Zool. Soc. Lond.* **123:** 551–561.

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THE BIOLOGICAL BULLETIN accepts original research reports of intermediate length on a variety of subjects of biological interest. In general, these papers are either of particular interest to workers at the Marine Biological Laboratory, or of outstanding general significance to a large number of biologists throughout the world. Normally, review papers (except for a limited number of solicited review papers which may be accepted after formal refereeing), very short papers (less than five printed pages), preliminary notes, and papers which describe only a new technique or method without presenting substantial quantities of data resulting from the use of the new method cannot be accepted for publication. A paper will usually appear within four months of the date of its acceptance.

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Continued on Cover Three

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EFFECTS OF EARLY STARVATION PERIODS ON ZOEAL DEVELOPMENT OF BRACHYURAN CRABS

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ABSTRACT

Larvae of the crabs *Menippe mercenaria* Say (Menippidae), *Panopeus herbstii* Milne-Edwards, *Neopanope sayi* Smith (Xanthidae), *Sesarma cinereum* Bosc (Grapsidae), and *Libinia emarginata* Leach (Majidae) were reared in the laboratory. Starvation periods different in length and timing within the first zoeal stage were studied as to their effects on later development and survival rate. After 1–3 days of initial feeding, most larvae had accumlated enough reserves to reach the second stage, independently of further food availability. The development of the survivors was delayed in the following stages, and their later mortality rate was higher than the fed controls. Starvation periods commencing directly after hatching of the larvae exert far stronger negative effects than those beginning later. All observations suggest a particularly sensitive phase in the beginning of larval life in brachyurans. When initial starvation periods exceed the point-of-no-return (PNR), the larvae will die later, even if feeding begins long before the energy reserves are depleted. Temporary lack of suitable prey may be an ecological factor controlling the survival of crab larvae as effectively as physical factors.

INTRODUCTION

The larvae of a great number of brachyuran crab species have been reared under different environmental conditions in the laboratory, especially under varying temperatures and salinities. However, few studies consider the influence of varying food levels, and even fewer investigated the effects of temporary absence of suitable food on later development and survival. As pointed out by Anger and Dawirs (1981), such periods of starvation should be expected in a natural, variable environment. Thus, experimental data gained in cultures with extremely high food densities (this applies to most of the literature) indicate the potential survival and development rate under close-to-optimum conditions, rather than realistic figures to be expected in nature.

Anger and Dawirs (1981) found in larvae of the spider crab *Hyas araneus* that temporary lack of prey during the first zoeal stage could exert different effects on survival and development rates of both zoeal instars. The kind and extent of these effects was related to the timing and duration of the starvation periods. Several of their observations were in good agreement with those reported in the literature (Kurata, 1959; Yatsuzuka, 1962; Modin and Cox, 1967; Kon, 1979; Paul and Paul,

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Abbreviations: Z-1, Z-2, zoeal stages 1, 2, etc.; PNR, point of no return (*sensu* Blaxter and Hempel, 1963): length of starvation after which a re-fed larva cannot recover; PRS, point of reserve saturation (*sensu* Anger and Dawirs, 1981): point at which larvae have enough reserves to molt independent of further food supply.

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1980). This correspondence suggested the existence of some general response patterns common to many decapod larvae exposed to temporary early starvation.

The present study attempts to find such general trends in zoeal development of brachyurans and to distinguish them from species-specific responses.

MATERIALS AND METHODS

In summer 1980, ovigerous females of five brachyuran species were collected near Beaufort, North Carolina, and brought to the Duke University Marine Laboratory. The five were *Menippe mercenaria* Say (Menippidae), *Panopeus herbstii* Milne-Edwards, *Neopanope sayi* Smith (Xanthidae), *Sesarma cinereum* Bosc (Grapsidae), and *Libinia emarginata* Leach (Majidae).

M. mercenaria and *L. emarginata* were kept in large running seawater tables until their larvae were almost ready to hatch. Then they were transferred to smaller aerated aquaria (*ca.* 5 l capacity), from which the larvae were isolated a few hours after hatching. The other species, which are smaller, were maintained in bowls with *ca.* 1 l seawater. Water was changed daily, and the crabs were not fed until the larvae hatched and were isolated.

Taking into consideration the place of origin of the ovigerous females, as well as tests reported in the literature, the following salinities were applied in incubating and rearing the larvae: 30‰ in *M. mercenaria* (*cf.* Ong and Costlow, 1970), 25‰ in *P. herbstii, N. sayi,* and *S. cinereum* (*cf.* Costlow *et al.*, 1960, 1962), and 35‰ in *L. emarginata* (*cf.* Johns and Lang, 1977). Many larvae of *P. herbstii* and *N. sayi* died, presumably due to unsuitable salinity, but also possibly because the hatches were not sufficiently viable. The experiment with larvae of *P. herbstii* was repeated later at 35‰ with better success. Salinities were obtained by mixing filtered seawater (Gelman glassfiber filter type A/E) with sufficient amounts of deionized freshwater, and were checked by means of a refractometer. All experiments were carried out at 25°C in constant-temperature cabinets, with a 12:12 L:D photoperiod.

Larvae were transferred individually with large-bore pipettes into numbered vials containing *ca.* 15–20 ml seawater and (where applicable) *ca.* 100–200 freshly hatched *Artemia salina* nauplii (San Francisco Bay Strain lot #1737). Approximately every 23–25 h, water and food were changed and molts or mortality of individual larvae were recorded. Experiments were finished when all larvae had either died or metamorphosed to the megalopa stage. No attempts were made to rear megalopa further. Larvae were considered dead when they were opaque, or when no movement of any appendage or interanl structure could be seen under moderate magnification.

Each experiment (one species) comprised eight sets (subexperiments) of 25 larvae each. In each subexperiment a different feeding regimen was tested:

1. Food was provided only during the first *ca.* 24 h following hatching. The larvae were then transferred to clean vials after being washed in baths of filtered seawater to avoid accidental transfer of food organisms. No further feeding was done until a larva successfully molted to the second stage.
2. Same as (1), but initial feeding period 2 days.
3. Same as (1), but initial feeding period 3 days.
4. No starvation (fed control).
5. Starvation only during first ca. 24 h after hatching; then feeding.
6. Same as (5), but initial starvation period 2 days.

7. Same as (5), but initial starvation period 3 days.
8. No feeding period (starved control).

If not otherwise stated, mean values of time spans are given as arithmetic mean ± 95% confidence intervals (the latter only when $n > 3$). Differences between mean values were tested after comparing their variances (F test) by means of Student's t statistic. They were considered statistically significant if p (two-tailed) was <0.05.

Mean durations of particular stages do not necessarily add to the cumulative figures (Tables I and II) unless there was no mortality.

The abbreviations Z-1, Z-2, etc. denote zoeal stages 1, 2, etc. Numbers of subexperiments (*e.g.* 4 = fed control) refer to the feeding regimens described above.

RESULTS

Rate of development and survival

Figures 1 and 2 give development and mortality rates for the first two zoeal stages of two representative species (*Menippe mercenaria* and *Panopeus herbstii*). The starved control group (8) is omitted, because no larvae reached the second stage.

Under condition 1 (1 day of initial feeding) only a few larvae of *Libinia emarginata* and *Neopanope sayi* reached the Z-2 stage. None survived to metamorphosis (megalopa).

Mortality figures for the Z-2 are given in Figures 1 and 2 as percentage of larvae which survived to this instar. Further data are presented in Tables I and II.

Menippe mercenaria

In the fed controls (4, Table I), 68% of the larvae reached megalopa stage after 17.85 ± 0.60 days. Starvation always delayed development and increased mortality as compared to this control group. Larvae initially fed for 2–3 days before starvation began had lowered survival in the Z-1 stage, but also slightly (statistically insignificant) shortened development to the second zoea. The Z-2, however, which was not starved itself, showed a significant prolongation (Fig. 1). Duration of Z-3 also increased (Table I).

When lack of prey occurred directly after hatching (subexperiments 5–7; Table I, Fig. 1), another pattern was observed: The Z-1 stage was significantly prolonged. After only 1 day of initial starvation, this delay was a little shorter than 1 day, but it was 3.55 days after 2 days starvation. In the latter subexperiment (subexperiment 6) mortality also strongly increased in the first two stages. After 3 days without food (subexperiment 7), no larva recovered after re-feeding.

Initial starvation in the Z-1 apparently caused a weak shortening of the duration of the following instar (Fig. 1). In the Z-3 and Z-4 some delay was noted (Table I), but it was statistically not significant.

Panopeus herbstii

The first experiment with *P. herbstii*, carried out at a salinity of 25‰, resulted in low survival, delayed development, and high variation in molt frequency. It remains in question whether this was due to unsuitably low salinity or low genetic viability of the hatch. The results are included in Table I because they still show a pattern:

In the fed control group (subexperiment 4) 64% of the larvae reached the second instar after 5.56 ± 0.76 days. Z-1 duration was shortened in subexperiments 2 and

TABLE I

M. mercenaria *and* P. herbstii: *Duration of zoeal stages and of cumulative development (cum.) in days (mean ± 95% confidence intervals), and mortality (%) given different feeding regimens (see text for details of regimens).*

		Feeding regimen																		
		1	2			3			4			5			6			7		
			days			days			days			days			days			days		
Species	Stage	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%
Menippe mercenaria	Z-1	100	3.50	0.75	72	3.50	0.37	20	4.09	0.54	12	4.91	0.79	32	7.64	0.99	72			100
	Z-2		4.67	0.54	4	3.92	0.33	8	3.42	0.24	12	3.07	0.39	8	3.00	0	12			
	cum.		8.00	1.22	76	7.75	0.55	28	7.24	0.25	24	7.70	0.48	40	10.25	0.80	84			
	Z-3		2.67	0.68	0	2.90	0.57	4	2.56	0.25	4	2.71	0.42	4	4.67		4			
	cum.		10.67	1.23	76	10.67	0.76	32	9.83	0.42	28	10.43	0.48	44	14.83		88			
	Z-4		3.33	0.54	0	3.75	0.82	0	3.29	0.24	4	3.71	0.56	0	3.67		0			
	cum.		14.00	1.10	76	14.42	1.42	32	13.21	0.51	32	14.14	0.86	44	18.50		88			
	Z-5		5.60	2.42	4	4.40	0.50	12	4.67	0.36	0	4.77	0.56	4	4.50		4			
	cum.		19.30	3.21	80	18.90	1.48	44	17.85	0.60	32	18.73	1.02	48	21.00		92			
Panopeus herbstii 25‰	Z-1	100	3.50		96	3.50	0	84	5.56	0.76	36	6.33	1.11	52			100			100
	Z-2				4	5.00		8	4.00		56	3.33		36						
	cum.				100	8.50		92	7.50		92	8.50		88						
35‰	Z-1	100	2.55	0.10	16	2.78	0.19	0	2.54	0.08	0	3.66	0.15	0	5.35	0.17	16	7.17		88
	Z-2		3.14	0.22	0	2.88	0.23	4	2.52	0.21	0	2.86	0.15	8	3.00	0.36	0	3.00		4
	cum.		5.69	0.27	16	5.67	0.30	4	5.06	0.21	0	6.54	0.16	8	8.35	0.27	16	10.00		92
	Z-3		3.05	0.18	4	2.96	0.36	4	2.79	0.25	4	3.11	0.29	20	3.00	0.36	16	3.00		0
	cum.		8.75	0.34	20	8.59	0.49	8	7.88	0.21	4	9.67	0.39	28	11.38	0.36	32	13.00		92
	Z-4		3.76	0.22	12	4.31	0.26	28	4.07	0.57	36	3.57	0.30	16	3.82	0.40	24	4.00		4
	cum.		12.56	0.34	32	12.75	0.53	36	11.90	0.69	40	13.29	0.52	44	15.14	0.34	56	17.50		96

TABLE II

N. sayi, S. cinereum, *and* L. emarginata: *Duration of zoeal stages and of cumulative development (cum.) in days (mean ± 95% confidence intervals), and mortality (%) given different feeding regimens (see text for details of regimens).*

		Feeding regimen																				
		1			2			3			4			5			6			7		
		days			days			days			days			days			days			days		
Species	Stage	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%	$\bar{x}$	±	%
Neopanope sayi	Z-1	4.50		96	3.28	0.34	64	3.41	0.36	56	4.13	0.42	16	5.88	0.62	68			100			100
	Z-2			4			36	5.00	0.88	24	4.41	0.37	16	4.00		20						
	cum.			100			100	8.50	0.88	80	8.21	0.57	32	9.50		88						
	Z-3							5.67	1.43	8	5.10	0.63	28	4.50		4						
	cum.							14.50		88	13.10	1.40	60	14.00		92						
Sesarma cinereum	Z-1			100	3.25	0.29	52	3.17	0.26	16	3.24	0.35	8	4.11	0.42	28	6.17	1.27	6	9.50		96
	Z-2				5.17	0.43	12	3.20	0.45	8	2.69	0.26	12	4.07	0.80	12	4.50	3.04	8	2.00		0
	cum.				8.67	0.43	64	6.70	0.45	24	5.94	0.22	20	8.03	0.83	40	10.25	2.72	84	11.50		96
	Z-3				3.16	0.54	0	3.20	0.45	0	2.94	0.31	8	3.73	0.99	0	4.50	2.05	0	5.00		0
	cum.				11.83	1.08	64	9.90	0.60	24	8.88	0.47	28	11.77	1.57	40	14.75	4.57	84	16.50		96
	Z-4				4.33	0.68	12	4.36	0.45	4	3.88	0.38	4	3.86	0.45	16	5.75	1.52	0	6.00		0
	cum.				15.50	2.39	76	14.20	0.68	28	12.70	0.93	32	14.86	1.09	56	20.50	4.31	84	22.50		96
Libinia emarginata	Z-1	3.43	0.27	44	2.92	0.24	24	2.60	0.14	16	2.62	0.18	36	3.67	0.25	52	6.04	0.46	56	6.50		96
	Z-2			56	4.14	0.35	48	3.40	0.28	24	3.27	0.31	20	4.00		40	4.50	0.57	20			4
	cum.			100	6.79	0.45	72	5.97	0.29	40	5.95	0.31	56	7.50		92	10.50	0.66	76			100

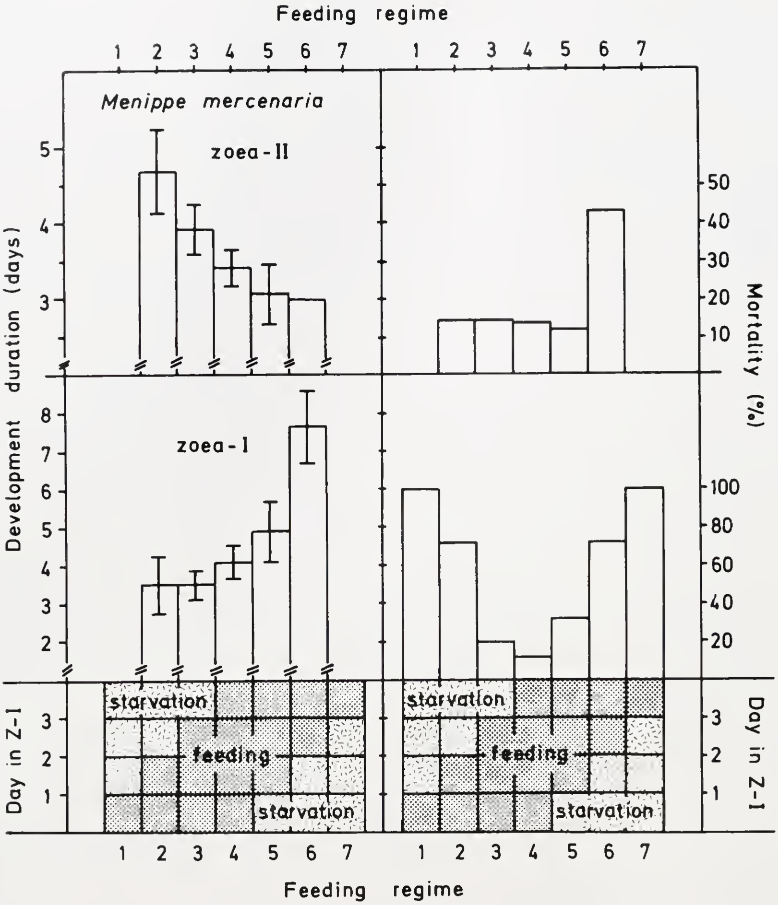

FIGURE 1. *Menippe mercenaria*: Development duration (days; mean ± 95% confidence intervals) and mortality (%) in the first and second larval instars in relation to the feeding regime (feeding/starvation schedule) during the zoea-1 stage.

3. Due to low survival, delay in Z-2 duration (as observed in *M. mercenaria* in these subexperiments) was only visible in feeding regimen 3: Z-2 lasted 1 day longer than in the fed control.

Initial starvation of 2–3 days (subexperiments 6 and 7) caused 100% mortality in the Z-1 stage. As in *M. mercenaria*, one day of starvation at the beginning of the first zoeal instar lowered survival and delayed Z-1 less than 1 day.

The second experiment with *P. herbstii* (35‰ salinity) yielded far better results: In the fed control group there was no mortality until stage 3. Only in the Z-4 instar did many larvae die when attempting to molt to the megalopa.

Starvation at the end of the Z-1 stage did not influence its duration (Table I; Fig. 2), but as in the last two experiments, it significantly lengthened the Z-2 stage. As in *M. mercenaria*, the Z-3 also was slightly (statistically insignificantly) lengthened.

Starvation beginning directly after hatching again caused a considerable, statistically significant lengthening of Z-1 duration (Fig. 2). However, unlike the case

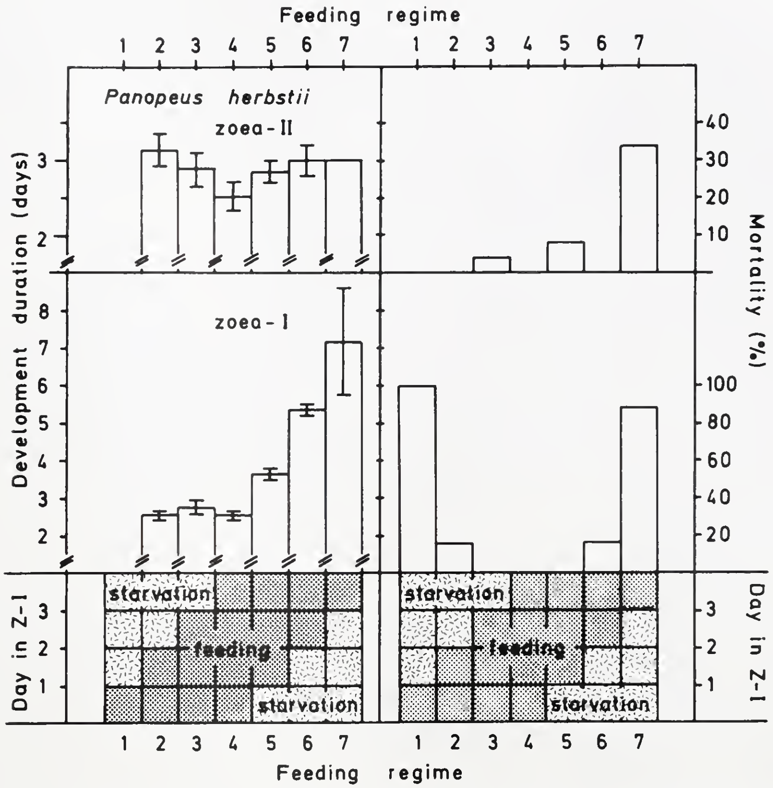

FIGURE 2. *Panopeus herbstii*: Development duration (days; mean ± 95% confidence intervals) and mortality (%) in the first and second larval instar in relation to the feeding regime (feeding/starvation schedule) during the zoea-1 stage.

in the previously described species, Z-2 was also significantly lengthened. Z-3 duration also was slightly prolonged. Since these effects are additive, even short initial lack of food caused a statistically significant delay in development to the megalopa. Mortality also was higher following initial starvation periods (Table I).

Neopanope sayi

As in the first experiment with *P. herbstii*, some factor caused high mortality in this species; none reached the megalopa stage (Table II).

Starvation during the first zoeal stage again increased mortality. Starvation at the end of this instar, did not clearly affect its duration, whereas the Z-2 was lengthened somewhat (Table II).

After 2–3 days of initial starvation, all larvae died in the Z-1. If this lack of food lasted for only 1 day, 32% of the larvae successfully molted to the Z-2 stage, with a delay of 1.75 days compared to fed controls. The few survivors reaching the next instar had a slightly shortened Z-2 development (4 days in contrast to 4.41 ± 0.37 in the control).

Sesarma cinereum

In fed controls (subexperiment 4) 68% of the larvae molted to the megalopa, 12.70 ± 0.93 days after hatching.

Survival and total development time were considerably affected by early starvation periods (Table II):

Duration of the Z-1 stage was not influenced by lack of food occurring at its end (Table II), but survival to the second zoea was lowered, and the Z-2 and Z-3 stages were prolonged.

In subexperiment 2, two different groups of larvae were considered separately:

1. Those that molted to Z-2 one day after commencement of starvation later did not show significantly delayed development nor any mortality in the Z-2 stage. Also, the Z-3 stage showed no difference from the fed controls.

2. Larvae that molted 2 days after commencement of starvation had a significantly prolonged Z-2, and their mortality increased. The Z-3 stage also was significantly prolonged (5.17 ± 0.43 vs. 2.94 ± 0.31 days in the fed control group).

In subexperiments in which the *S. cinereum* larvae were starved initially (5–7), the Z-1 stage was significantly prolonged and mortality increased (Table II), as in the species discussed previously. The duration of the Z-2 did not clearly change. However, all later zoeal stages became longer in relation to longer starvation periods. As in all species previously discussed, starvation periods had a far stronger effect when they occurred right after hatching, rather than later.

Libinia emarginata

The mortality pattern in larvae of the spider crab *L. emarginata* was veiled by high death rates in the beginning of the experiment. Development duration, was as in most cases, more strongly correlated to early starvation (Table II). Both zoeal stages were significantly prolonged due to lack of food, regardless of the timing of starvation. This effect, however, was again much more conspicuous when starvation took place in the beginning of the Z-1.

As in *S. cinereum*, the survivors of subexperiment 2 were considered in two groups according to their time of molting to the Z-2 stage: Only one out of eight larvae that had molted to the Z-2 during the fourth day after hatching (*i.e.*, after 2 days starvation) later successfully molted to the megalopa. In the other group, which had reached the Z-2 during the third day, six out of eleven larvae later became megalopae.

Mortality patterns

Four types of mortality may be distinguished:

1. Initial mortality: During the first 2 days of experiments, death rates were often relatively high regardless of feeding regimens. This type of mortality, strongest in *L. emarginata*, is rather unpredictable. It is related to low viability of individual larvae unable to adjust to the experimental conditions.

2. Mortality before and during molting: Larvae normally have higher death rates during molting periods than during intermolt. Short starvation periods at the beginning of the Z-1 stage (subexperiments 5 and 6), often increased this type of mortality drastically. It was most conspicuous in *P. herbstii, S. cinereum,* and *L. emarginata*.

3. Mortality after re-feeding: Larvae initially starved for 2–3 days often had high death rates during the first 2 days following feeding. This effect was obvious in all species except *S. cinereum*; it was strongest after 3 days of initial lack of prey (subexperiment 7). *M. mercenaria, P. herbstii* (first experiment), and *L. emarginata* larvae treated this way had significantly shorter mean survival times

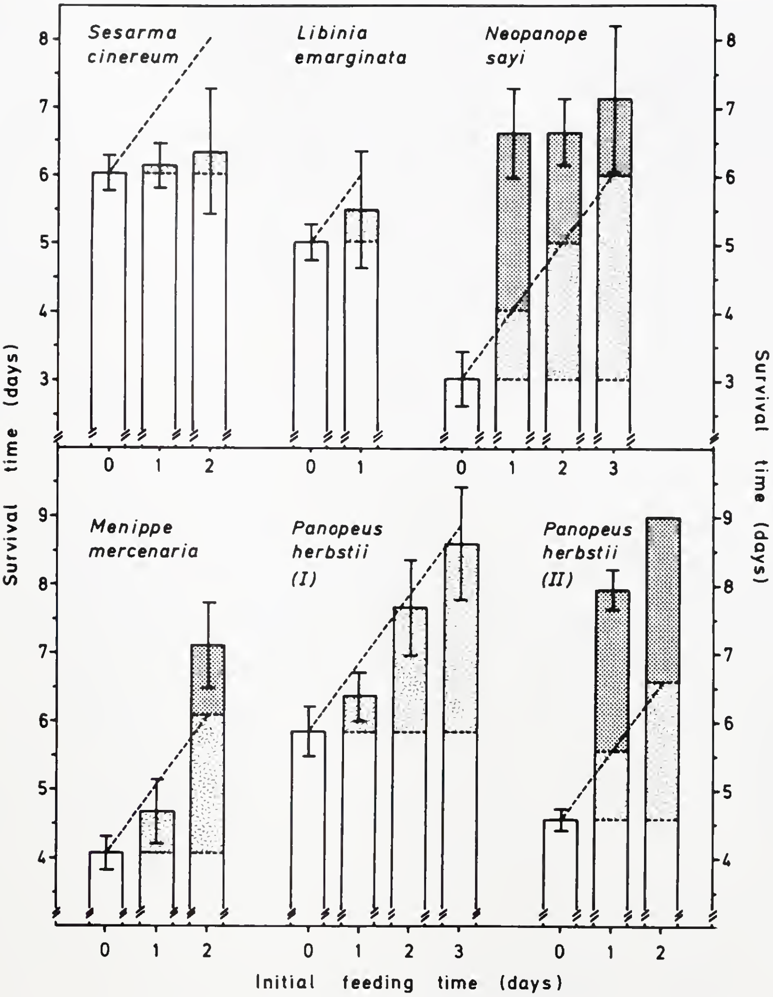

FIGURE 3. Survival time (days; mean ± 95% confidence intervals) in crab larvae (first stage) starved after different feeding periods. Dotted lines: Survival time in the starved control (0 days) plus duration of feeding period. For further explanation see text.

than those in the completely starved control (subexperiment 8). The figures were 3.42 ± 0.35 versus 4.08 ± 0.25 days in the first species, 5.04 ± 0.35 versus 5.86 ± 0.36 days in the second, and 4.27 ± 0.36 versus 5.02 ± 0.26 days in the third.

4. Death due to depletion of reserves: In the starved control group, larvae died mostly in well-defined periods of time. These periods of time could be used to show the detrimental effect of too late re-feeding (see above) or to test the larvae's food uptake afte starvation: In *N. sayi*, for example, it did not make any difference whether the larvae were re-fed after 2 or 3 days or never. The survival times were not significantly different, *i.e.*, the larvae must have lost their ability to catch or to eat prey. The same effect was observed in *S. cinereum* in subexperiment 7.

The starved control group made possible comparison (Fig. 3) of the mean survival time in those larvae which were fed for some time before starvation (subexperiments 1–3), but did not reach the Z-2 stage, with the sum of the survival time under complete starvation (subexperiment 8) plus the initial feeding period (represented by dotted lines in Fig. 3). Three effects were observed:

1. Survival was prolonged approximately by the time span of initial feeding, *i.e.*, during each day of feeding enough reserves were accumulated to survive 1 later day of starvation (slightly shaded parts of bars in Fig. 3). This type of response was found in *P. herbstii* (first experiment) and in subexperiment 1 in *M. mercenaria* and *L. emarginata.*
2. Survival was prolonged by more than the feeding period (dark shaded part of bars in Fig. 3), indicating a comparatively fast rate of reserve accumulation. This pattern was observed in *P. herbstii* (second experiment), *N. sayi*, and in part in *M. mercenaria.*
3. Initial feeding periods did not significantly influence survival time under later starvation. Only in *S. cinereum* was such weak accumulation of reserves found.

DISCUSSION

If a marine environment is variable, and characterized by temporary lack of suitable prey (patchiness in time and space) then our observations have an important ecological implication: Even short starvation periods in early larval development of brachyurans can severely affect their later chance of survival. These negative effects, such as increased mortality and delayed development, might be at least as significant as those exerted by variations in temperature and salinity.

The present study concerned whether general effects were common to brachyuran larvae subjected to temporary starvation. Some lethal and sublethal effects are summarized in Figure 4. To average all observed effects exerted on development duration, a "development factor" was calculated for every feeding regimen. This index sets the stage durations in the fed control (subexperiment 4) at 1.0. If, for example, a given stage in a subexperiment is prolonged to double the control figure, the development factor is 2.0; if it was shortened to 80% of the control value, then the index is 0.8. Mean values and standard deviations were obtained in this way from all experiments (Fig. 4). Figures represented by single individuals only were omitted from this presentation. The mortality values in the graph were arc-sin transformed before averaging.

Short starvation at the end of the Z-1 did not strongly affect the duration of this instar. In *L. emarginata* it significantly prolonged the stage, but in the other species it had no effect or shortened the stage. The longer this starvation (*i.e.*, the shorter the initial feeding period) the higher was mortality in Z-1. In some species (*cf.* Table II), the stage tended to be longer when starvation commenced after only 1 day of feeding. Anger and Dawirs (1981) found a similar pattern in *Hyas araneus*: Short periods without food at the end of the Z-1 stage appeared to slightly shorten this stage, whereas long periods lengthened it. This effect is too weak and may depend too much on the species (see *L. emarginata*) for speculation on its causes without further observations.

The experimental design for subexperiments 1–4 was too rough to determine exact values for the point-of-reserve-saturation (PRS_0; Anger & Dawirs, 1981). In most species considered here it was more than 1, but less than 2 days. Only in *L. emarginata* was even the PRS_{50} below 1 day, *i.e.* 50% of the larvae had accumulated enough reserves at this time to molt successfully to the Z-2 stage without

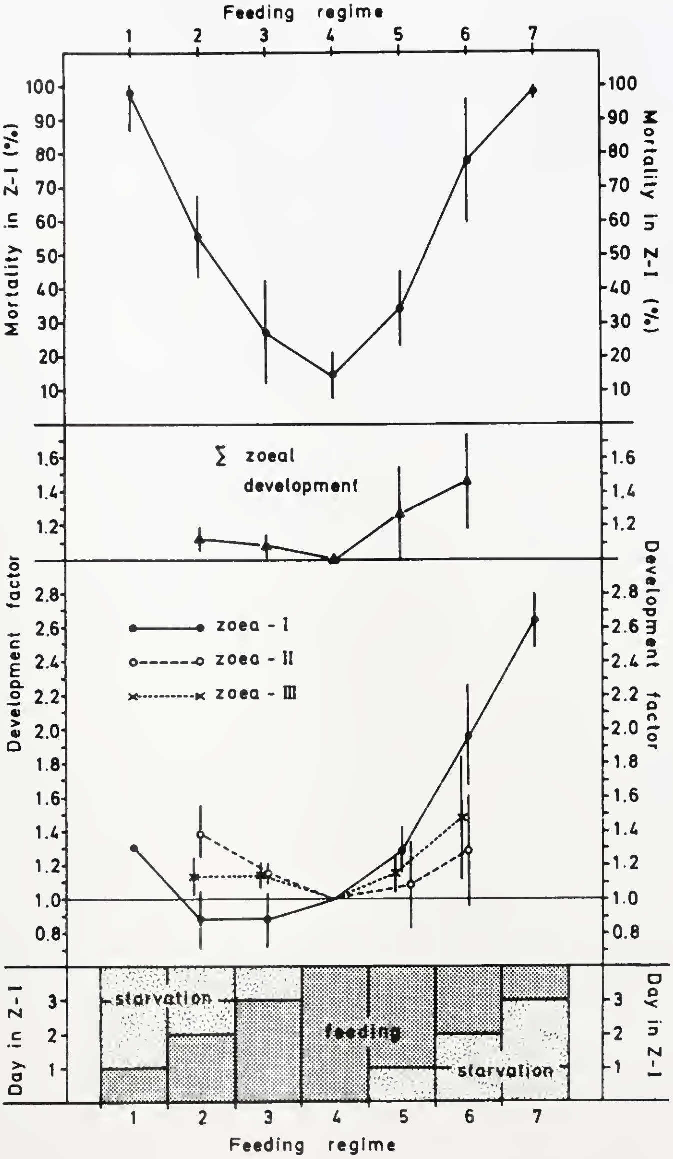

FIGURE 4. Change in development duration, expressed as multiple of fed control (= feeding regimen no. 4), and mortality (%) in zoeal development of crab larvae in relation to feeding/starvation schedule during the first stage: Averaged effects from all experiments (mean ± standard deviation).

further food supply (Table II). The mean PRS_{50} value of all species was *ca.* 2 days (Fig. 4).

The amount of reserves accumulated during early feeding periods probably is not only species-dependent, but is also influenced by other factors (Fig. 3): In the first experiment with *P. herbstii*, during each day of feeding a reserve for about

1 day of future starvation was accumulated. In the second experiment, much more reserves were stored.

Individual variability also plays a role: As shown in subexperiment 2 in *S. cinereum* and *L. emarginata*, larvae that molted sooner than others to the Z-2 stage were much less affected by starvation before the molt. Though the same age, they apparently had developed faster and consequently were further advanced when starvation commenced, giving them a better chance of survival than slower-growing siblings.

In all experiments in this study, starvation commencing toward the end of the Z-1 stage significantly prolonged the Z-2, and to a lesser degree the following stage (Fig. 4). The same effect was found by Anger and Dawirs (1981) in *H. araneus* larvae. In the present study, death rates during the Z-2 instar also increased in most cases where the Z-1 had been starved before molting (Table II). Since the lengthening in later stages was longer than the shortening of Z-1, starvation toward the end of Z-1 caused, on an average, a weak prolongation in total zoeal development (Fig. 4).

Any starvation period at the beginning of the Z-1 caused a highly significant lengthening of this stage in all species considered (Figs. 1 and 2; 4). The same was observed in other brachyuran species by Kon (1979), Anger and Dawirs (1981), and by Dawirs (unpublished). This effect is apparently a general response pattern: Zoeal development apparently does not begin to use existing reserves from the egg, unless some essential cue is provided by food taken up. Anger and Dawirs (1981) discussed possible mechanisms in early larval reconstruction processes and suggested that sterols, precursors of the molting hormone ecdysterone, may play a crucial role as a starting cue. As in the present study, they found that the delay was approximately equivalent to the duration of the starvation period in Z-1 larvae of *H. araneus*. This indicates that except for chitin synthesis (see Anger and Nair, 1979) developmental processes awaited the starting signal given by first feeding. When the time span without food supply increased, the delay corresponding to this period was further increased, during which time lost reserves may have had to be replenished.

In all experiments of this type, a "point-of-no-return" (PNR; for recent discussion see Anger and Dawirs, 1981) was observed, mostly long before the energy reserves were ultimately depleted. The time when 50% of the starved larvae could not recover when being re-fed (PNR_{50}) was short in the species investigated here: Mostly it was *ca.* 1–2 days, *i.e.* considerably shorter than the survival time under continuous starvation (cf. Fig. 3). The high difference between the PNR levels in the two experiments with *P. herbstii* (Table I) that showed this measure of starvation resistance probably is also influenced by other factors, such as perhaps salinity. Early starvation periods lasting 1–3 days strongly increased deaths in the first zoeal stage (Fig. 4). Later survival also tended to decrease (Figs. 1 and 2). The influence of such starvation immediately following hatching was apparently always stronger than where a feeding period preceded starvation (subexperiments 1–3).

The effects of initial lack of prey on Z-2 duration were not uniform: In *P. herbstii, S. cinereum,* and *L. emarginata* a lengthening was found, but in the other two species a slight shortening. The latter effect was also observed by Anger and Dawirs (1981) in *H. araneus* larvae. Surprisingly, in all species of the present study there was some prolongation of the Z-3 stage, and in some instances even of the Z-4 (Tables I and II). The effect in the Z-3 was on an average even stronger than the delay in the Z-2 (Fig. 4). As a consequence of these patterns, the whole zoeal development to the megalopa was clearly delayed. These effects of initial starvation were much stronger than those caused by later fasting (Fig. 4): When suitable prey was absent during only the first 48 h of larval life, zoeal development was delayed at an average by almost 50%, and almost 80% of the larvae did not even reach the second instar.

Our results confirm a particularly critical period in the beginning of larval development of brachyuran crabs (Kon, 1979; Anger and Dawirs, 1981) and perhaps also in other decapods (Kurata, 1959; Modin and Cox, 1967; Paul and Paul, 1980). The latter authors observed that early starvation in king crab zoeae lowered ability to catch prey after re-feeding. This effect was probably responsible for the similar survival times of re-fed and continuously starved larvae of some species in the present study. However, when initial fasting was short, survival usually was significantly prolonged in larvae not reaching the second stage (as compared to the starved control). This indicates that the larvae were still able to catch, ingest, and convert food, but had lost their ability to molt successfully. Anger and Dawirs (1981), who observed the same effect in spider crab larvae (*H. araneus*), suggested an irreversible damage to some hormonal or enzymatic system controlling molt. Biochemical studies combined with experiments as designed for the present investigation should explain the mechanisms involved. Future studies will also have to extend to later larval stages (especially to the megalopa) and to interactions of starvation with other ecological and biological factors.

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LITERATURE CITED

ANGER, K., AND K. K. C. NAIR. 1979. Laboratory experiments on the larval development of *Hyas areneus* (Decapoda, Majidae). *Helgoländer Wiss. Meeresunters.* **32:** 36–54.

ANGER, K., AND R. R. DAWIRS. 1981. The influence of starvation on the larval development of *Hyas areneus* (Decapoda, Majidae). *Helgoländer Wiss. Meeresunters.* **34:** 287–311.

COSTLOW, J. D., JR., C. G. BOOKHOUT, AND R. MONROE. 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biol. Bull.* **118:** 183–202.

COSTLOW, J. D., JR., C. G. BOOKHOUT, AND R. MONROE. 1962. Salinity-temperature effects on the larval development of the crab, *Panopeus herbstii* Milne-Edwards, reared in the laboratory. *Physiol. Zool.* **35:** 79–93.

JOHNS, D. M., AND W. H. LANG. 1977. Larval development of the spider crab *Libinia emarginata* (Majidae). *Fish. Bull.* **75:** 831–841.

KON, T. 1979. Ecological studies on larvae of the crabs belonging to the genus *Chionoecetes*. I. The influence of starvation on the survival and growth on the Zuwai crab. *Bull. Jpn. Soc. Sci. Fish.* **45:** 7–9.

KURATA, H. 1959. Studies on the larva and post-larva of *Paralithodes camtschatica.* I. Rearing of the larvae, with special reference to the food of the zoea. *Bull. Hokk. Reg. Fish. Res. Lab.* **20:** 76–83.

MODIN, J. C., AND K. W. COX. 1976. Postembryonic development of laboratory-reared ocean shrimp, *Pandalus jordani* Rathbun. *Crustaceana* **13:** 197–219.

ONG, K., AND J. D. COSTLOW, JR. 1970. The effect of salinity and temperature on the larval development of the stone crab, *Menippe mercenaria* (Say), reared in the laboratory. *Chesapeake Sci.* **11:** 16–29.

PAUL, A. J., AND J. M. PAUL. 1980. The effect of early starvation on later feeding success of king crab zoeae. *J. Exp. Mar. Biol. Ecol.* **44:** 247–251.

YATSUZUKA, K. 1962. Studies on the artificial rearing of the larval Brachyura, especially of the larval blue crab, *Neptunus pelagicus* Linnaeus. *Rep. USA Mar. Biol. Stn.* **9:** 1–88.

Reference: *Biol. Bull.* **161**:213–235. (October, 1981)

SPAWNING, DEVELOPMENT, AND ACQUISITION OF ZOOXANTHELLAE BY *TRIDACNA SQUAMOSA* (MOLLUSCA, BIVALVIA)

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ABSTRACT

Spawning and development of the clam *T. squamosa* was investigated in Belau, Western Caroline Islands in February and March 1980. Five of the six species of tridacnid clams spawned in response to various stimuli, but only *T. squamosa* released mature eggs. Factors influencing gonad development and spawning are discussed.

Development of *T. squamosa* was followed from post-fertilization to post-metamorphosis, with emphasis on the acquisition of the zooxanthella *Symbiodinium (=Gymnodinium) microadriaticum* Freudenthal. The symbiotic algae were not seen in either the fertilized eggs or trochophore stages. We found that all strains of *S. microadriaticum* introduced to veliger clams were taken into the stomach via the mouth. Veligers ingested motile zooxanthellae more readily than non-motile ones. Within 2–9 days *after* metamorphosis, zooxanthellae moved by an unknown mechanism into the developing siphonal tissues. Most of these zooxanthellae appeared to be in spaces, probably the developing haemal sinuses. However, in some cases it was difficult to tell if the zooxanthellae were intra- or extracellular. Survival and growth of veligers and juveniles with zooxanthellae was greater than those without zooxanthellae. Juveniles with zooxanthellae can survive and grow in Millipore-filtered seawater with light as the sole energy source for over 10 months, illustrating the phototrophic aspect of the association.

Our observations may have practical application pertinent to spawning, development, and growth of tridacnid clams in the context of commercial mariculture.

INTRODUCTION

There has been much interest in the biology of tridacnid clams, particularly in regard to their symbiosis with algae, since Yonge's (1936) classic study. The tridacnids and the heart shell *Corculum cardissa*, members of the Superfamily Cardiacea, are the only marine bivalves known to harbor endosymbiotic dinoflagellates (Brock 1888, Boschma 1924, Yonge 1936, Kawaguti 1950, 1968). These algae have been identified as *Symbiodinium (=Gymnodinium) microadriaticum* (Freudenthal, 1962) (hereafter also referred to as zooxanthellae), allegedly the same species of dinoflagellate that inhabits all reef-building corals and many anemones, gorgonians, hydroids, and jellyfishes (*cf.* Freudenthal 1962, Taylor 1969, 1974; Loeblich and Sherley, 1979; Schoenberg and Trench, 1980a, b, c). The zooxanthellae in tridacnids are most numerous in the hypertrophied tissues of the clams' siphon, but may also be found in the heart, stomach, digestive gland, and intestine (Mansour 1946a, b, c; Kawaguti 1968; Goreau *et al.*, 1973; Trench *et al.*, 1981).

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Yonge (1936) hypothesized that the algae were probably passed from parents to offspring. However, subsequent studies by LaBarbera (1975) and Jameson (1976) have shown that neither eggs nor sperm released from tridacnids contain zooxanthellae, implying that each generation must acquire their complement of symbionts from the environment.

The latter observations raise a number of intriguing questions. First, when are the zooxanthellae acquired during the development of the clam? Larval tridacnids maintained in unfiltered seawater and observed just after metamorphosis already had zooxanthellae in their siphon tissues (LaBarbera, 1975; Jameson, 1976). Whether these algae were acquired before or after metamorphosis, or are needed for metamorphosis, was not determined. Dependence of an organism on a symbiont for completion of its life cycle may be more common than originally thought (see Provosoli *et al.*, 1968; Taylor, 1971b; Edson, 1981). Other examples are *S. microadriaticum* and the rhizostome jellyfish *Mastigias papua* (Sugiura, 1963, 1964) and *Cassiopeia xamachana* (Trench *et al.*, 1981; Trench, 1981).

Second, what is the mechanism of acquisition of the algae? In the scyphistomae of *C. xamachana,* zooxanthellae are taken in via the mouth and are phagocytozed by endoderm cells (Trench, 1980; Trench *et al.*, 1981). The zooxanthellae take up permanent residence inside the digestive cells of the coelenterate and somehow avoid digestion by the host (Trench, 1979). The situation in the Tridacnidae is somewhat different. Zooxanthellae in the siphon tissues are not found inside cells. Instead they lie free in the blood sinuses. Morton (1978) suggested that infection might be by "accidental invasion" of the clam, the zooxanthellae somehow moving through the "external surface of the mantle". The other possible route is through the digestive system. It was originally thought that this was unlikely because of the efficient digestive capabilities of herbivorous filter-feeding bivalves (Yonge, 1936; 1975). However, several studies have shown that some algae can pass through the digestive system of bivalves without being digested (Coe, 1948; Dean, 1958; Haven and Morales-Alamo, 1966; Hildreth, 1980). In the Tridacnidae, apparently healthy zooxanthellae (Mansour, 1946b, c), including motile forms (Ricard and Salvat, 1977) capable of fixing $^{14}CO_2$ and being cultured (Trench *et al.*, 1981) are commonly extruded in fecal pellets.

Do tridacnid clams establish a symbiosis only with certain species or strains of zooxanthellae? Three species of dinoflagellates have been described as endosymbionts in marine invertebrates; two have been placed in the genus *Amphidinium* and the remaining one is referred to as *S. microadriaticum* (Freudenthal, 1962; Taylor, 1971a, 1973, 1974). A vast majority of the symbiotic coelenterates contain the latter symbiont. Distinct strains of *S. microadriaticum* have recently been distinguished by biochemical and morphological criteria (Schoenberg and Trench, 1980a, b). Selectivity in the uptake of strains of *S. microadriaticum* has been experimentally documented (Schoenberg and Trench, 1980c; Trench 1981; Trench *et al.*, 1981). Tridacnid clams are found in symbiotic associations *only* with *S. microadriaticum* (Taylor, 1969). This observation, coupled with the fact that naturally occurring aposymbiotic adult clams have never been reported, suggests selectivity in the process of acquisition leading to specificity in the association. Discrimination during the uptake of different strains of *S. microadriaticum* by the Tridacnidae has not been previously addressed.

In this study we investigated these three questions concerning the acquisition of *S. microadriaticum* by larval and juvenile *T. squamosa.* We found that all strains of *S. microadriaticum* introduced to veliger clams were taken in via the mouth. They made their way by an unknown mechanism into the siphonal tissues *after* metamorphosis. Motile zooxanthellae were more apt to be taken into veliger stomachs and may lead to higher veliger survival than non-motile zooxanthellae. Survival and growth of veligers and juveniles was greater with zooxanthellae than without them.

MATERIALS AND METHODS

Experimental organisms

Adult tridacnid clams, except for *Tridacna gigas,* were collected from their natural habitat on the reefs of Belau, Western Caroline Islands, and transported in containers of sea water to the Micronesian Mariculture Demonstration Center (MMDC) on the Island of Malakal. Specimens of *T. gigas* were collected in 1978–1979 by personnel at the MMDC and were living on the reef adjacent to the laboratory. All individuals of the other five species were maintained in outdoor cement tanks in unfiltered running seawater until spawning experiments began.

Release of gametes

Three methods of initiating spawning were attempted: introduction of macerated gonads from a clam of the same species, placing clams in warm water, and introduction of hydrogen peroxide. In addition, spontaneous (non-induced) spawnings were documented. Water flow in the tanks was stopped during spawning experiments. All attempts on *T. gigas* were performed *in situ.*

We attempted to induce spawning with macerated gonads the day after collection and, in some cases, after periods ranging from 1 week to 1 month after collection. In practice, portions of gonad were dissected from one individual, macerated, and scattered in tanks of water containing other members of the same species. For induction of spawning with warm water we set clams in 30 l fiberglass tubs and let the water heat up to 30–35°C in the sun for periods ranging from 1–8 h. Small clams were placed in containers with 12 l of seawater and spawning was induced with 7.5×10^{-2} M hydrogen peroxide brought to pH 9.1 with the addition of 2M TRIS (see Morse *et al.*, 1977). For larger clams, 6% hydrogen peroxide was squirted directly into the incurrent siphon. The actual concentration reaching the clam was not determined.

Fertilization

Freshly spawned eggs were obtained by rinsing a clam spawning eggs in seawater and placing it in a tub of 20 l of seawater uncontaminated by sperm. The clam was allowed to go through 1–3 spawning reactions (see Wada, 1954) before being moved back to the cement holding tank. Sperm-laden water (~50 mls), derived from other clams, to minimize the chance of self fertilization, was added to the container of eggs and mixed thoroughly (see LaBarbera, 1975).

Maintenance of larvae

Fertilized eggs were pipetted into sterile Petri dishes containing 0.22 μm Millipore-filtered seawater (MFSW). All subsequent developmental stages were maintained in MFSW in the laboratory, at ambient air temperatures (24–31°C) and illumination. Seawater was changed approximately every 4 days. Except for zooxanthellae, there were no other additives to the cultures.

TABLE I

Host sources of isolates of zooxanthellae taken into the gut of veligers of Tridacna squamosa. *Strain designations correspond to those of Schoenberg and Trench (1980a). "U," uncharacterized.*

Cultured	(Class, Phylum)	Strain
Cassiopeia xamachana	(Scyphozoa, Coelenterata)	C
*Zoanthus sociatus**	(Anthozoa, Coelenterata)	Z
*Zoanthus solanderi***	(Anthozoa, Coelenterata)	U
Aiptasia tagetes	(Anthozoa, Coelenterata)	A
Aiptasia pallida	(Anthozoa, Coelenterata)	U
Tridacna gigas	(Bivalvia, Mollusca)	T
Tridacna maxima	(Bivalvia, Mollusca)	U
Freshly Isolated		
Tridacna squamosa	(Bivalvia, Mollusca)	U
Tridacna gigas	(Bivalvia, Mollusca)	U
Tridacna maxima	(Bivalvia, Mollusca)	U
Tridacna derasa	(Bivalvia, Mollusca)	U
Tridacna crocea	(Bivalvia, Mollusca)	U
Hippopus hippopus	(Bivalvia, Mollusca)	U
Aiptasia sp.	(Anthozoa, Coelenterata)	U
Mastigias papua	(Scyphozoa, Coelenterata)	U

* Strain "Z," isolated in Jamaica in 1974 by D. A. Schoenberg.
** Strain "U," isolated in Jamaica in 1979 by S. S. Chang.

Introduction of zooxanthellae

Previously cultured and freshly isolated zooxanthellae were introduced to cultures of veligers 2 days after spawning (see Table I). Strains of cultured *S. microadriacticum* were originally isolated from various invertebrate hosts, including sea anemones, jellyfishes, and two species of *Tridacna*. They were grown in the artificial culture medium ASP-8A (McLaughlin and Zahl, 1957, 1959, 1966; Ahles, 1967) for at least 1 year before use in experiments. Approximately 0.2 ml wet-packed cells were added to Petri dishes of veligers. Freshly isolated zooxanthellae were from all six tridacnid clams, a local anemone, *Aiptasia* sp., and the jellyfish *Mastigias papua*. Freshly isolated zooxanthellae were obtained by scraping a piece of clam siphon or jellyfish manubrium tissue with a scalpel in a Petri dish of MFSW until a brown suspension made up of released zooxanthellae and small pieces of animal tissue covered the bottom. This suspension was allowed to stand for 5 min before the supernatant, containing animal tissue, mucus, and some zooxanthellae, was poured off. Zooxanthellae adherent to the bottom of the Petri dish were rinsed gently with MFSW, squirted off the bottom, centrifuged, and combined until approximately 0.2 ml of wet-packed cells were accumulated. Algal pellets released from *Aiptasia* sp. were collected, washed twice with MFSW, and centrifuged to a 0.2 ml wet-packed pellet before use in infection experiments.

Microscopy

A light microscope with a calibrated occular micrometer was used to measure the length (longest dimension) of the larval and juvenile *Tridacna* and to determine presence or absence of zooxanthellae. Some sorting and veliger counts were made using a dissecting microscope.

Clams were fixed for electron microscopy in Karnovsky's (1965) fixative for 1 h, rinsed twice with 0.2 *M* sodium cacodylate buffer (pH 7.3), and post-fixed with 1% osmium in 0.2 *M* sodium cacodylate buffer for 1 h. Specimens were stored in 70% ETOH at 4°C prior to preparation for transmission electron microscopy (TEM). Specimens were dehydrated in ETOH and transferred through 5 changes of propylene oxide before being embedded with Araldite 6005. Sections were made on a LKB Ultramicrotome V with glass knives, stained with uranyl acetate and lead citrate, and observed and photographed on a Siemens Elmiskop I.

RESULTS

Spawning

All three artificial methods induced spawning, but none produced viable eggs (Table II). However, two instances of spontaneous spawning of *T. squamosa* produced viable eggs. These occurred on consecutive days (2–3 March 1980) in the morning, coincident with a new moon and a falling tide, in a group of clams collected 3 weeks earlier. Spawning began at 1000 h and continued for over 9 h. Sperm were produced first, for up to 6 h with much individual variation. This was followed by the release of eggs 3–9 h after the beginning of sperm release. All individuals (n = 55) in the tank spawned sperm. However, the number of clams releasing eggs was not determined.

The addition of macerated gonads to water containing adult clams almost always induced spawning with release of sperm within 1–5 min. Release of sperm continued 0.5–6 h. Many of the clams thus tested showed weak spawning behavior, producing low concentrations of sperm over a 2 h period. *T. derasa* spawned in response to the addition of hydrogen peroxide. Within 15 min these clams reacted to the addition of 6% hydrogen peroxide into the incurrent siphon with vigorous discharge of sperm and the production of large amounts of mucus, continuing 4–6 h. Dissections showed large gonads with many sperm but no mature eggs.

TABLE II

Number of spawning events for four methods of induction during the period February 19 to March 17, 1980. s = sperm spawned, e = eggs spawned, n = number of attempts. W = Winter, Sp = Spring, Su = Summer, F = Fall.

			Method											
			Macerated gonads			Elevated temperature			Peroxide			Spontaneous		
Species	Reported breeding season	Total number tested	s	e	n	s	e	n	s	e	n	s	e	n
Tridacna squamosa	W[1]	55	3	0	4	0	0	1	0	0	1	3	2	3
Tridacna maxima	W[2]	50	4	0	4	1	0	2	0	0	1	1	0	1
Tridacna derasa	Sp[3]	15	1	0	1	—	—	—	1	0	2	—	—	—
Tridacna crocea	Su[4]	40	0	0	1	0	0	2	0	0	2	1	0	1
Tridacna gigas	F[5]	6	0	0	1	—	—	—	0	0	1	—	—	—
Hippopus hippopus	Su[6]	14	2	0	2	—	—	—	—	—	—	1	0	1

[1] Wada (1954), Rosewater (1965), Hardy and Hardy (1969), LaBarbera (1975), Beckvar (1981).

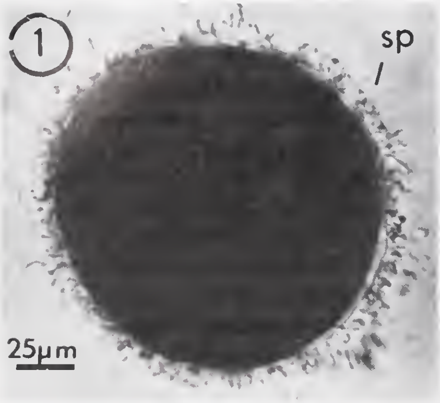
[2] LaBarbera (1975), Jameson (1976).

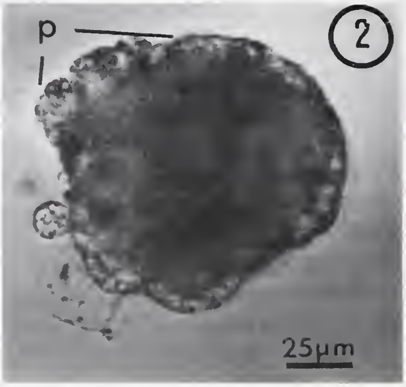
[3] Jameson (1976), Beckvar (1981).

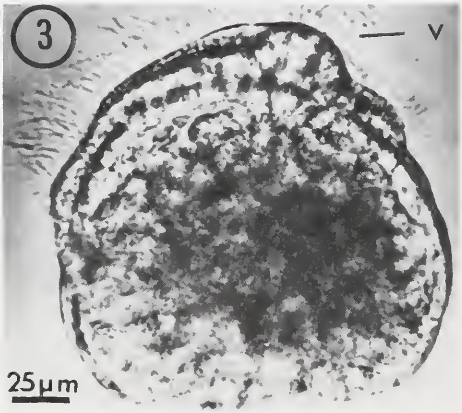
[4] Jameson (1976).

[5] Beckvar (1981).

[6] Stephenson (1934), Jameson (1976), Yamaguchi (1977), Beckvar (1981).







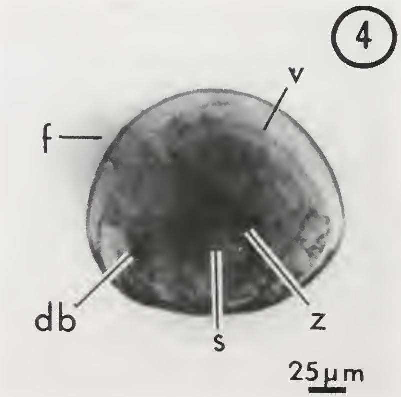


FIGURE 1. Egg spawned from *T. squamosa*. Note sperm (sp) around the outside of the egg.

FIGURE 2. Trochophore larva of *T. squamosa*. A band of cilia, known as the prototroch (p), is visible at the anterior end of the animal.

FIGURE 3. Day 3 veliger of *T. squamosa*. v = velum.

FIGURE 4. Pediveliger of *T. squamosa. The foot* (*f*) *and velum* (*v*) are both present at this stage of development. Zooxanthellae (z) can be seen lining the stomach. Usually 3–4 dark bodies (db) are present in the posterior portion of the animal.

Induction of spawning by raising the water temperature was generally unsuccessful. Clams tended to close and release mucus as water temperature rose, indicating unsatisfactory conditions.

Larval development

Fertilized eggs (Fig. 1) passed through the 8–16 cell stage in 3–6 h. Ciliated gastrulae appeared 6–9 h post-fertilization. Trochophore larvae (Fig. 2) were first observed between 12–20 h after fertilization. Veligers (Fig. 3) appeared 24–30 h after fertilization. Two days after fertilization veligers had a well developed velum, a stomach and an esophagus. The intestine at this point is still a solid cylinder

(LaBarbera, 1975), developing a lumen on day 3. Pediveligers (Fig. 4) appeared on day 10, and alternately swam and crawled on the bottom for at least a day before metamorphosis. There was extreme variation in times of pediveliger development; some were observed up to 29 days after fertilization (see Table V). No zooxanthellae were observed in any of these developmental stages in animals maintained in MFSW.

Infection with algal symbionts

Cultured and freshly isolated *S. microadriaticum* (see Table I) were introduced into Petri dishes of veligers on day 2. Zooxanthellae were not seen in the alimentary tract of the veligers until the end of day 3. All strains of zooxanthellae tested were seen in the stomachs of the clams during the veliger stages, except those derived from pellets extruded by the local *Aiptasia* sp. (Table III). The different strains of zooxanthellae exhibited different degrees of maximum motility (cf. Fitt *et al.*, 1981). A comparison of motility levels and presence of zooxanthellae taken into the stomach (Table III) suggested that these two phenomena might be related.

To test whether motile zooxanthellae are more likely than non-motile zooxanthellae to be ingested by veligers, the following experiment was performed. Twenty uninfected veligers were placed in petri dishes of zooxanthellae from *T. squamosa* during the day when motile forms were present. After 7 h, 90% of the veligers had zooxanthellae in their stomachs. Only 10% of veligers had zooxanthellae in their stomachs when the same experiment was done at night, when no motile zooxanthellae were present. To establish if these differences were due to nocturnal changes in feeding behavior of the veligers, the experiment was repeated with cultured zooxanthellae isolated from *A. pallida*. This alga is motile in the morning and non-motile in the afternoon (Fitt *et al.*, 1981). Results corroborated the first experiment. Between 0600 h and 1300 h, 90% of the 20 uninfected veligers ingested zooxanthellae; while between 1400 h and 2100 h, only 10% of the veligers were found with zooxanthellae in their stomachs.

To determine if different strains of zooxanthellae affect growth of larval clams, we measured shell length over time. Measurements of day 8 veligers and pediveliger

TABLE III

Relative motility of zooxanthellae and the frequency of observations (% days) of zooxanthellae in the gut of veligers of Tridacna squamosa. *Relative maximum motility in seawater is designated by: + = less than 1% motile cells, ++ = 1–25%, +++ = 25–50%, ++++ = >50%. Observations on gut contents were begun on day 4 and ended when metamorphosis began on day 11. See text for further details.*

Host source of cultured zooxanthellae	Motility	% Days	Host source of freshly isolated zooxanthellae	Motility	% Days
Cassiopeia xamachana	+++	100	*Tridacna squamosa*	++++	100
*Zoanthus sociatus**	+	88	*Tridacna gigas*	+++	100
*Zoanthus solanderi***	++	100	*Tridacna derasa*	++	100
Aiptasia tagetes	+	25	*Tridacna crocea*	++	100
Aiptasia pallida	+++	100	*Tridacna maxima*	++	100
Tridacna gigas	++	100	*Hippopus hippopus*	++++	100
Tridacna maxima	++	100	*Aiptasia* sp.***	+	0

* Strain "Z." ** Strain "U." *** <10 motile cells seen during all observations.

TABLE IV

Length (µm) of veligers (day 8) and pediveligers of Tridacna squamosa *maintained in Millipore-filtered seawater with various isolates of zooxanthellae. SD = standard deviation.*

Host sources of zooxanthellae	Day 8 veligers			Pediveligers			
	Mean	SD	n	Mean	SD	n	Age (days)
Cultured							
Cassiopeia xamachana	158	6	10	169	13	31	11–29
*Zoanthus sociatus**	158	9	10	166	7	15	10–18
*Zoanthus solanderi***	153	9	10	—	—	—	—
Aiptasia tagetes	160	—	1	167	9	19	11–20
Aiptasia pallida	158	6	10	169	9	45	11–26
Tridacna gigas	162	6	10	161	10	19	11–17
Tridacna maxima	156	4	10	160	9	19	11–19
Freshly Isolated							
Tridacna squamosa	158	7	10	162	9	19	10–20
Tridacna gigas	—	—	—	165	5	13	11–17
Tridacna maxima	—	—	—	162	9	19	11–17
Tridacna derasa	—	—	—	167	6	19	10–17
Tridacna crocea	—	—	—	171	8	14	10–20
Hippopus hippopus	—	—	—	169	8	15	11–15
Aiptasia sp.	157	5	10	—	—	—	—
Mastigias papua	—	—	—	168	4	2	11–12
Control (no zooxanthellae)	152	6	10	159	7	14	11–20
Range	152–162			159–169			

* Strain "Z." ** Strain "U."

stages showed no significant ($p > 0.05$) differences in length between animals exposed to different strains of *S. microadriaticum* (Table IV). Veligers and pediveliger larvae (without zooxanthellae) were typically smaller than animals with zooxanthellae, although the differences were not significant.

Different strains of zooxanthellae were added to Petri dishes of 600 uninfected veligers on day 3. Survival was monitored on days 7, 8, 9, and 10 by determining the proportion of live and dead animals from a subsample of 30 animals. Veligers exposed to zooxanthellae freshly isolated from *T. squamosa* had significantly ($p < 0.05$, comparison of slopes) higher survival rates than veligers exposed to cultured zooxanthellae from *A. pallida, C. xamachana, Zoanthus solanderi, T. gigas,* and *T. maxima* (Fig. 5). Animals with the above strains of zooxanthellae all had significantly ($p < 0.05$, comparison of means) higher survival after 7 days than veligers with zooxanthellae isolated from *Z. sociatus* (strain Z), *A. tagetes* and the controls (without zooxanthellae). The experiment was terminated on day 10, when pediveligers began metamorphosing to juvenile clams.

Metamorphosis in the Tridacnidae is a gradual process, involving less swimming and more crawling as the shell gets heavier and the velum degenerates. The appearance of a statocyst at the base of the foot (Fig. 12) indicates the completion of metamorphosis (Jameson, 1976). The first juveniles were seen on day 11 (Table V). Minimum time to metamorphosis of veligers exposed to different strains of zooxanthellae was typically 11–15 days. However, there was wide variation in the times of development (see Table IV). Metamorphosis occurred in some clams as late as day 30. Clams without zooxanthellae metamorphosed at the same times as those with zooxanthellae. Mortality was high for all groups of clams, regardless of strain of zooxanthellae. Less than 5% of the veligers alive on day 3 survived through to complete metamorphosis, regardless of the experimental conditions.

No zooxanthellae were seen in the mantle region of newly metamorphosed clams (Fig. 6), in spite of the fact that zooxanthellae were common in the stomachs of

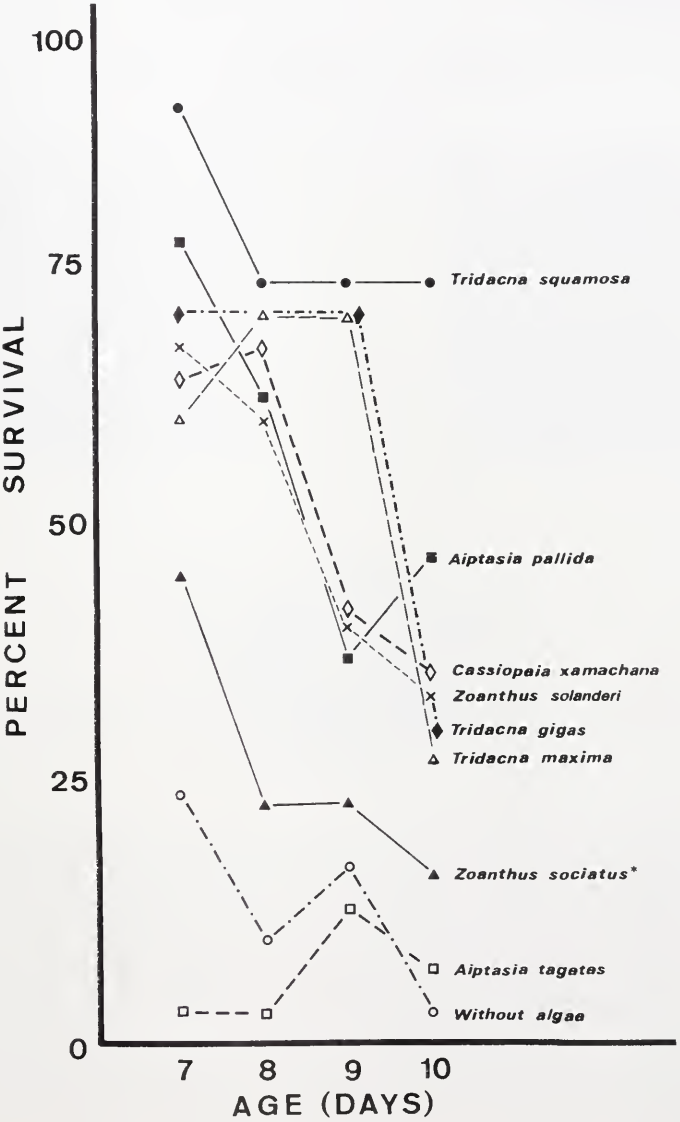


FIGURE 5. Percent survival of veligers of *T. squamosa* over 7 days in Petri dishes containing zooxanthellae isolated from the various hosts indicated on the right of each line. The experiment was terminated on day 11, when metamorphosis began. * = strain "Z", ** = strain "U".

TABLE V

Developmental timetable of late veliger and juvenile Tridacna squamosa *maintained in Petri dishes of cultured and freshly isolated zooxanthellae from various hosts. Numbers indicate minimum age in days.*

Host source of zooxanthellae	Pediveliger	Juvenile	Zooxanthellae in siphon tissues	Days between first juvenile sighting to first sighting of zooxanthellae in siphon tissue
Cultured				
Cassiopeia xamachana	11	14	18	4
*Zoanthus sociatus**	10	11	18	7
*Zoanthus solanderi***	11	—	—	—
Aiptasia tagetes	11	20	20	0***
Aiptasia pallida	11	15	18	3
Tridacna gigas	11	15	17	2
Tridacna maxima	11	15	18	3
Freshly Isolated				
Tridacna squamosa	10	13	17	4
Tridacna gigas	11	15	17	2
Tridacna maxima	11	11	20	9
Tridacna derasa	10	14	17	3
Tridacna crocea	10	14	16	2
Hippopus hippopus	11	14	19	5
Control (no zooxanthellae)	11	17		

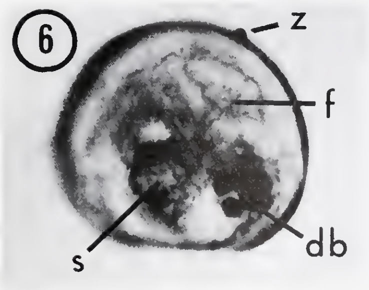
* Strain "Z."

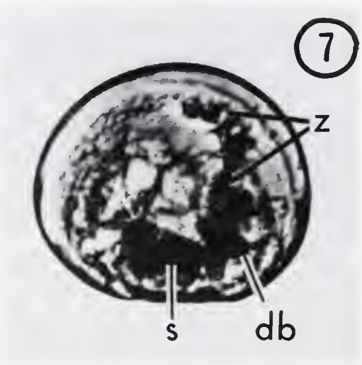
** Strain "U."

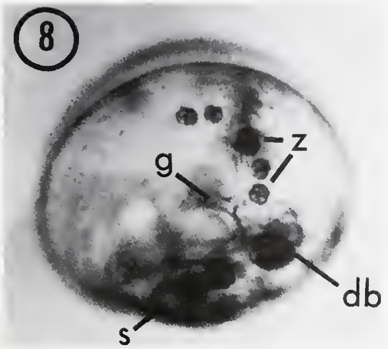
*** Only 1 juvenile survived metamorphosis; it was first found with zooxanthellae half-way across well-developed siphon tissues, indicating that metamorphosis took place several days previous to this observation.

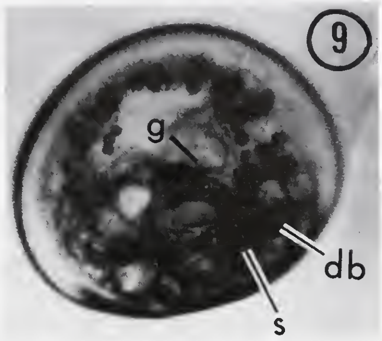
these clams. Between 2–7 days after metamorphosis (Table V) zooxanthellae appeared in the ventral mantle regions of the animal as two rows of cells, one on either side of the clam (Figs. 7, 11). This line of algae usually passed between or just anterior to three to four dark bodies located just posterior to the digestive gland and stomach/style sac (Figs. 7, 8). These zooxanthellae appeared healthy and many were seen dividing (Fig. 8). It took 1–3 weeks for the zooxanthellae to spread throughout the siphon tissue (Figs. 9, 12). This phenomenon was the same, regardless of the strain of zooxanthellae present.

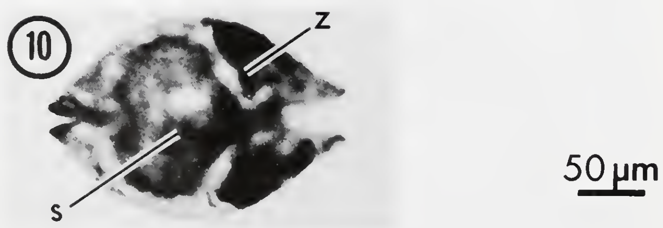
Electron microscopy did not reveal any clear differences in morphology or location of different strains of zooxanthellae in juvenile clams. Zooxanthellae in the stomach did not appear to be inside cells (Fig. 11); some appeared healthy while others showed signs of degeneration. It is not known whether unhealthy zooxanthellae were being digested or undergoing autolysis (*cf.* Fankboner, 1971; Muscatine, 1973; Trench *et al.*, 1981). Zooxanthellae were observed outside the











FIGURES 6–9. Hinge (dorsal) is down, anterior is left. Bar = 50 μm. FIGURE 6. Newly metamorphosed juvenile of *T. squamosa.* Zooxanthellae (strain, "T", isolated from *T. gigas*) can be seen inside the clam as dark spheres in the stomach (s) and outside of the clam next to the shell. There are no zooanthellae in the ventral mantle regions of the clam. Other symbols as in Fig. 4. Figure 7. A row of zooxanthellae (strain "U", isolated from *Z. solanderi*) in a 19-day-old *T. squamosa* juvenile, extending from the posterior stomach (s) region to the ventral mantle portion of the clam. Other symbols as in Fig. 4. Figure 8. Dividing zooxanthellae (strain "T", isolated from *T. gigas*) in the mantle tissues bordering the gills (g) of a 19-day-old *T. squamosa.* Other symbols as in Fig. 4. Figure 9. Zooxanthellae (strain "C", isolated from *C. xamachana*), extending across the entire ventral margin of a 21-day-old *T. squamosa.* Other symbols as in Figs. 4, 8. Figure 10. A dorsal view of a 19-day-old *T. squamosa* juvenile showing the relation between the heart-shaped stomach (s) lined with dark spherical zooxanthellae, and the rows of zooxanthellae on both sides of the posterior portion of the clam. Bar = 50 μm.

stomach either immediately adjacent to the stomach (Fig. 13), and the base of the foot (Fig. 12), or in the mantle tissues bordering the shell (Figs. 11, 14). These algae were probably in developing digestive diverticula and haemal sinuses. Most

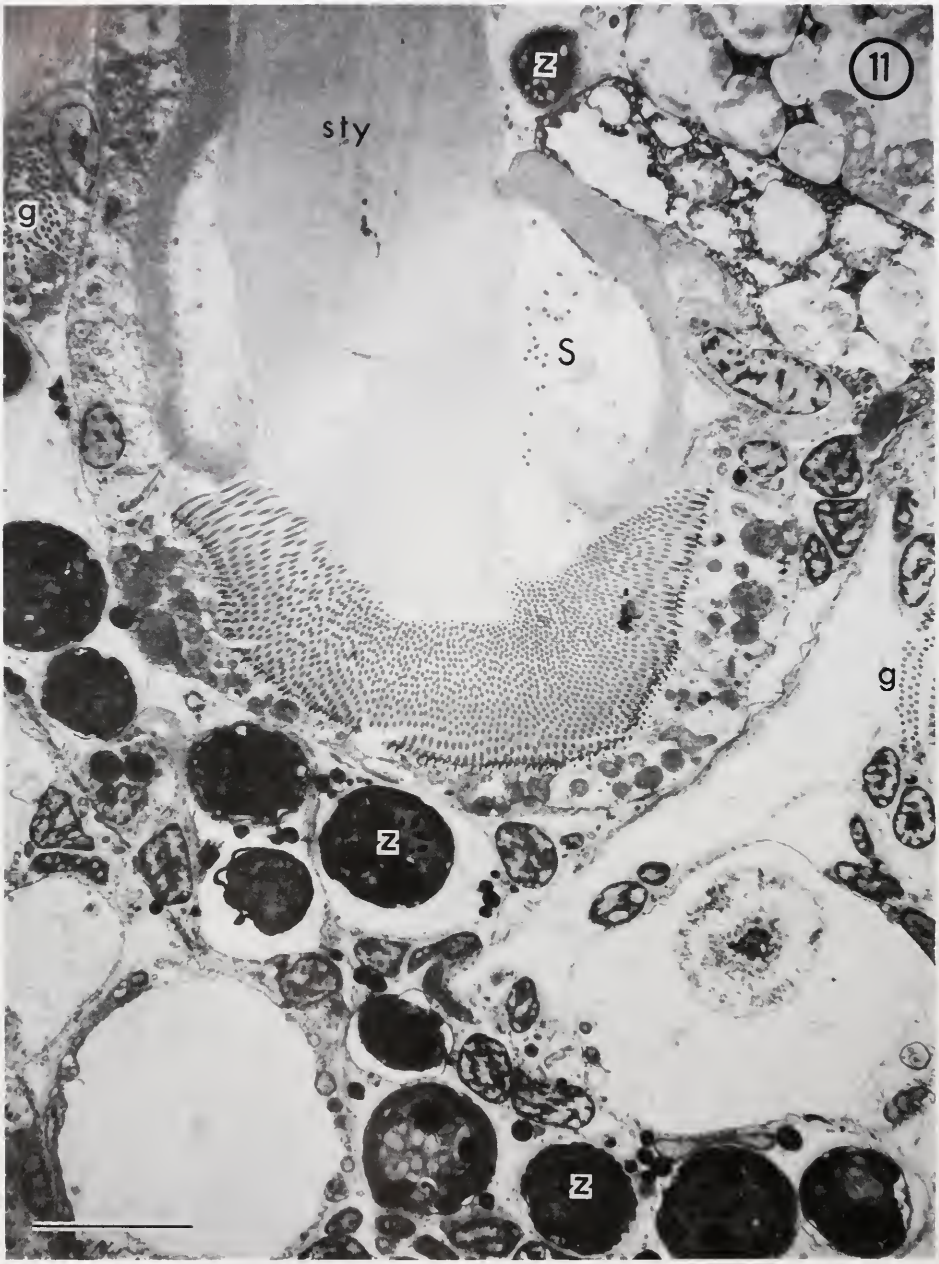
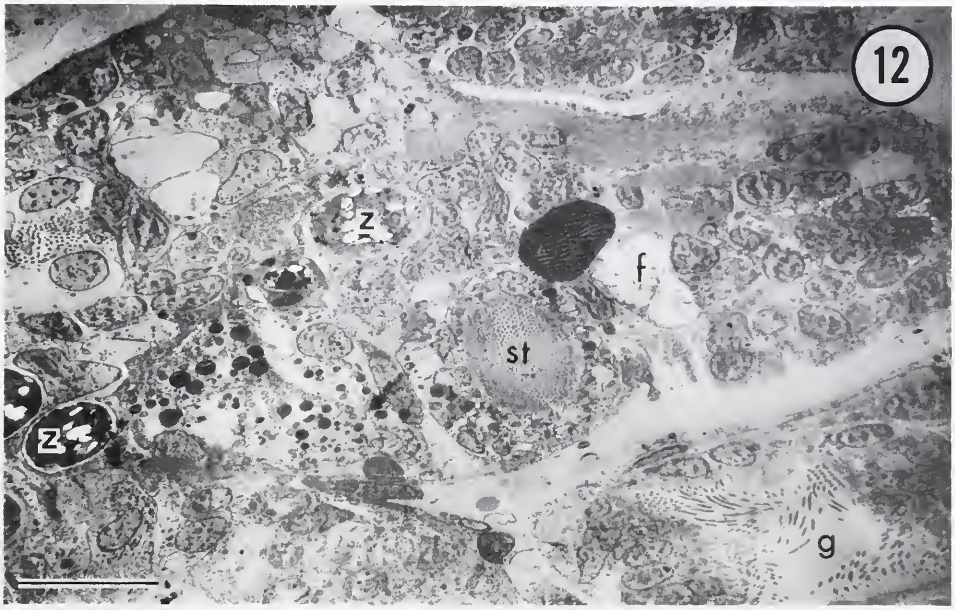


FIGURE 11. Oblique longitudinal section of a 20-day-old *T. squamosa* juvenile, showing the stomach (s) containing the style (sty) and a zooxanthella (z). Two rows of zooxanthellae extend from an area just posterior to the stomach to the left and right mantle regions of the clam just posterior to the gills (g). All zooxanthellae are strain "U", isolated from *A. pallida*. Bar = 10 μm.



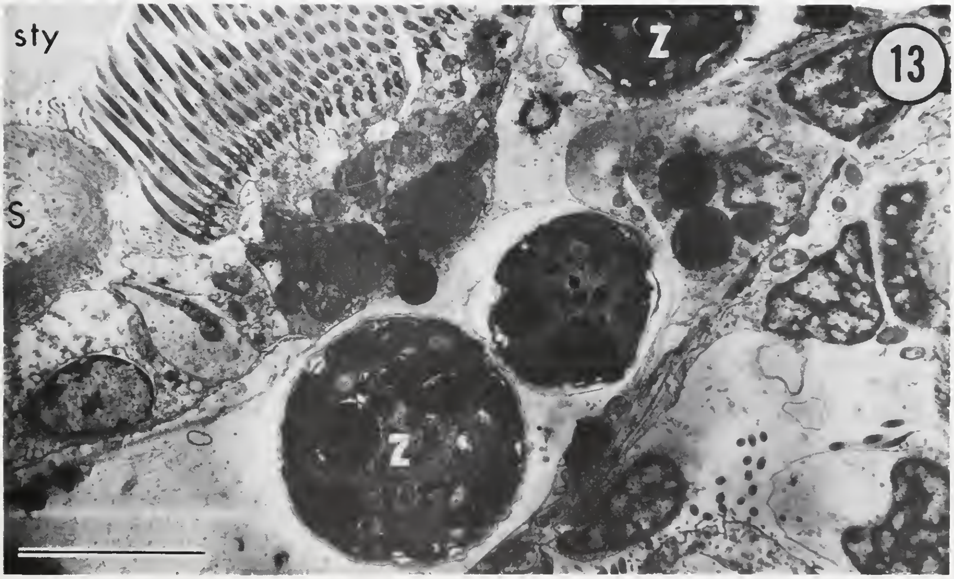
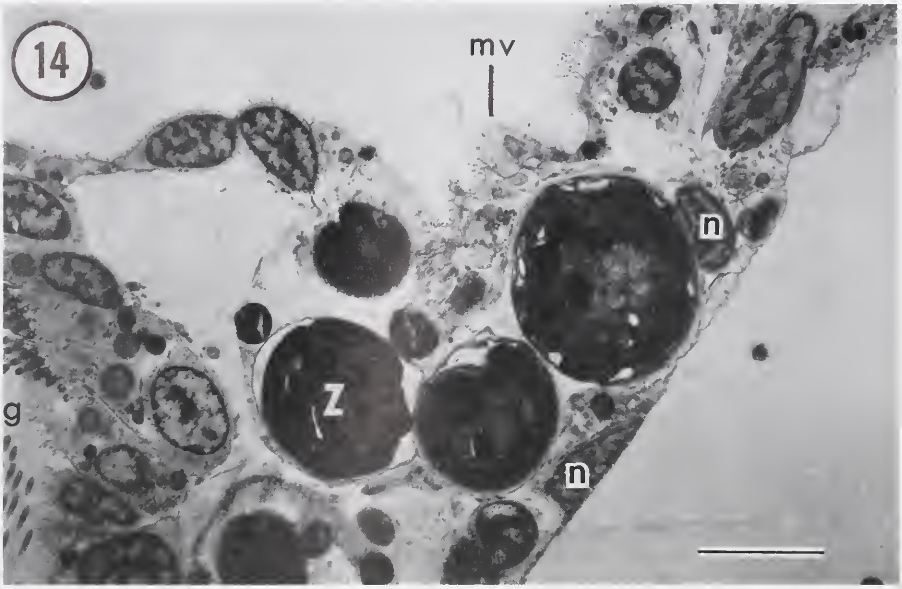


FIGURE 12. Transverse section of a 16-day-old *T. squamosa* juvenile with zooxanthellae (z) (strain "U", isolated from *T. crocea*) in the dorsal stomach region at the left and at the base of the foot (f) near the statocyst (st). A portion of the gills (g) is visible on one side of the clam. Bar = 10 μm.

FIGURE 13. Zooxanthellae (strain "U", isolated from *A. pallida*) in an open space (probably developing digestive diverticulae or haemal sinuses) just posterior to the stomach (s) in a 20-day old juvenile *T. squamosa*. Other symbols as in Fig. 11. Bar = 5 μm.



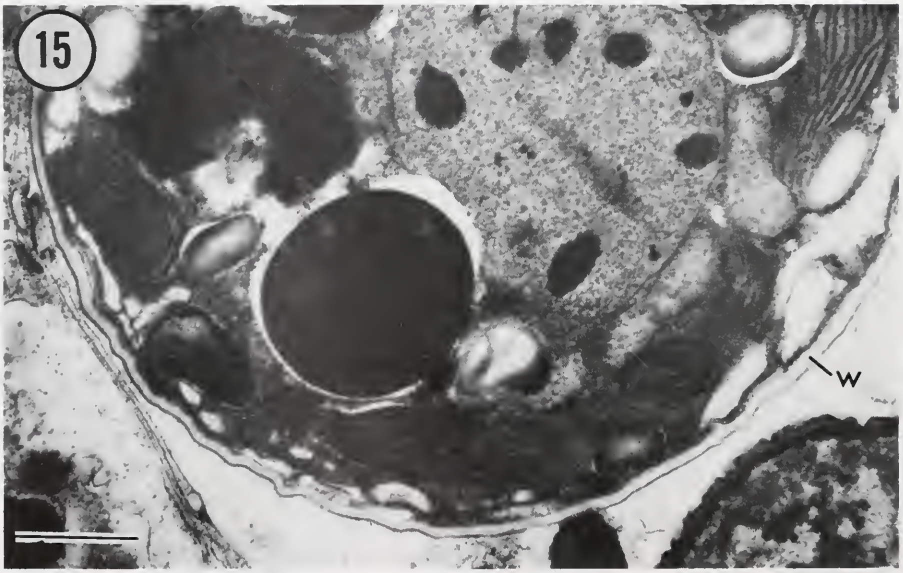


FIGURE 14. Zooxanthellae (z) (strain "U", isolated from *A. pallida*) in the mantle region of a 20-day-old juvenile *T. squamosa.* Note border of microvilli (mv) and close approximity of host cells and host cell nuclei (n). Bar = 5 μm.

FIGURE 15. A zooxanthella (strain "U", isolated from *A pallida*), lying free, probably in developing haemal sinuses in the mantle region of a 20-day old juvenile *T. squamosa.* w = zooxanthellar cell wall. Bar = 1 μm.

of these zooxanthellae appear to lie free in a space or tubule lined with host cells (Figs. 13, 14, 15). In some cases, however, it is difficult to tell from electron micrographs if the zooxanthellae are extra- or intracellular (Figs. 16, 17). Some of the zooxanthellae outside the stomach also appeared degenerate.

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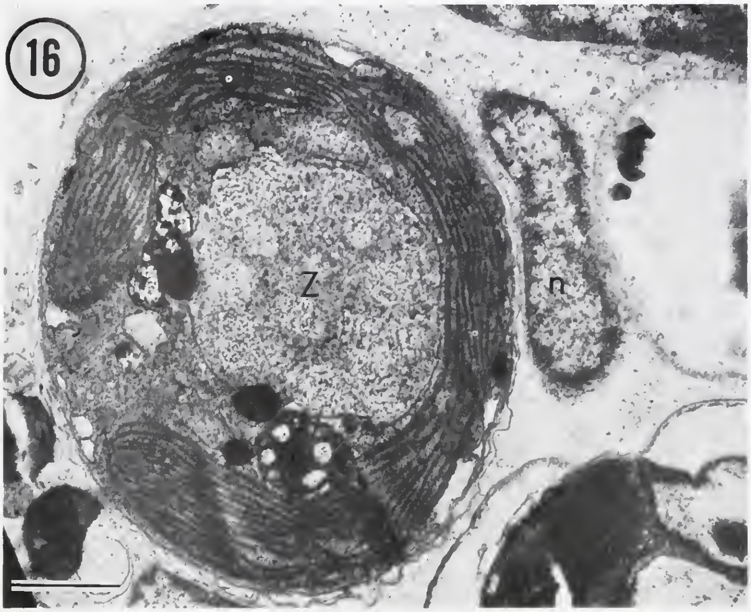

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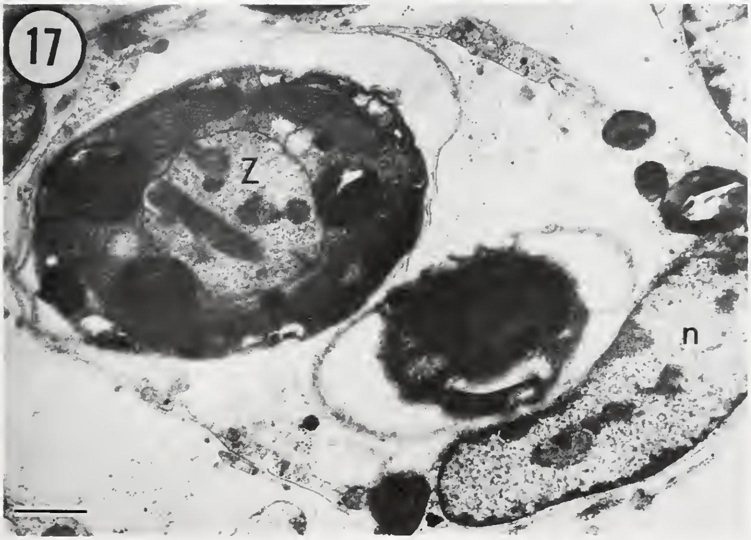

FIGURE 16. A zooxanthella (z) (strain "U", isolated from *A. pallida*) in the mantle region of a 20-day-old juvenile *T. squamosa* showing surrounding host tissue and a host nucleus (n). Bar = 1 μm.

FIGURE 17. A zooxanthella (z) (strain "U", isolated from *A. pallida*) apparently surrounded by a host cell in the mantle region of a 20-day-old juvenile *T. squamosa*. Other symbols as in Fig. 16. Bar = 1 μm.

Juveniles without zooxanthellae did not live beyond 3 weeks. Juveniles with zooxanthellae lived substantially longer; about 15 clams increased in length between 4–9 μ/day when maintained in MFSW in our laboratory over a 10 month period with light (60 $\mu E \cdot m^{-2} \cdot sec^{-1}$) as the sole known energy source.

Newly metamorphosed clams with zooxanthellae had growth rates comparable

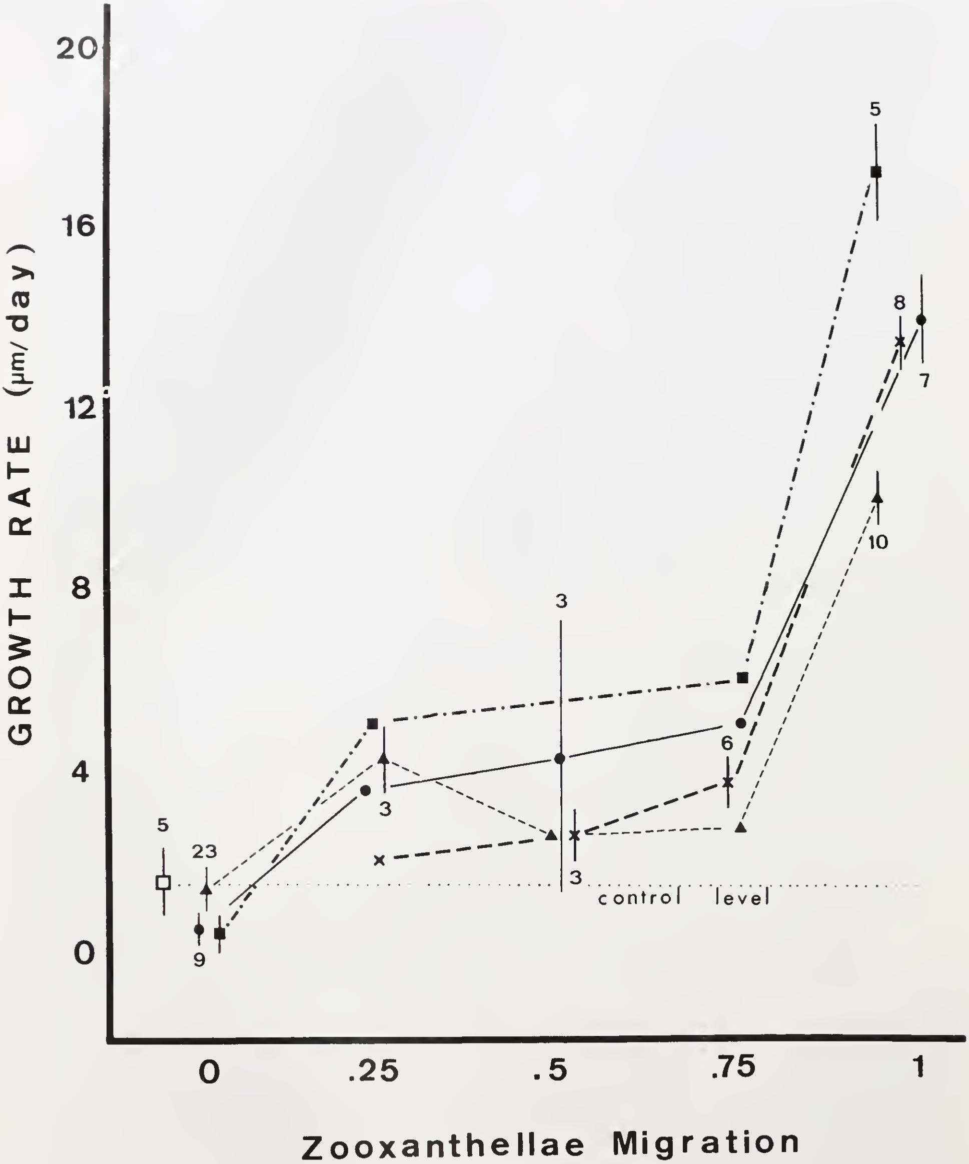

FIGURE 18. Growth rates of juvenile *T. squamosa* with different strains of zooxanthellae extending across their mantle regions. Scale for zooxanthellae migration: 0 = no zooxanthellae in mantle region (see Fig. 6), 0.5 = zooxanthellae extend about half way across ventral region of clam (see Fig. 7), 1 = zooxanthellae extend entirely across ventral portion of the clam (see Fig. 9). Clams contained zooxanthellae isolated from *H. hippopus* (■), *T. squamosa* (●), *A. pallida* (×), and *T. crocea* (▲). Control clams without zooxanthellae (□). Bars represent ±1 standard error, with "n" noted on one side of the bar.

to control clams without zooxanthellae. As the zooxanthellae moved across the central portion of the clam with the developing siphon tissues, the growth rates of the clams with zooxanthellae increased (Fig. 18). When zooxanthellae had moved completely across the siphon tissues, growth rates were significantly ($p < 0.05$) higher than controls, ranging from 6-20μm per day, and were comparable to those obtained by Jameson (1976). At this point, juveniles of *T. squamosa* infected with different isolates of zooxanthellae demonstrated different growth rates.

DISCUSSION

Spawning

The term "spawning" in bivalves usually refers to the release of sperm and/or eggs. Since tridacnids are hermaphroditic (Wada, 1952), spawning can refer to either the release of sperm or eggs, or both, from the same clam. Unfortunately, published accounts of tridacnid spawning often do not distinguish between these alternatives. In our experiments, we observed spawning in five of the six species of tridacnid clams. However, only 2 of the 31 spawning events recorded included the release of viable eggs after the release of sperm.

Spawning of tridacnid clams has been induced by the addition of macerated gonads (Wada, 1954; Rosewater, 1965; Hardy and Hardy, 1969; LaBarbera, 1975; Jameson, 1976), raising the temperature of the ambient water (Jameson, 1976; W. Hamner, pers. com.), and by hydrogen peroxide (Beckvar, 1981). Of these three methods, macerated gonads seems to be the most reliable, usually eliciting spawning within 5 min. Peroxide and warm water produce signs of stress in the clams, such as the production of mucus and closing of the shell. Other bivalves have been induced to spawn with chemical and thermal stimulation (see Loosanoff and Davis, 1963). In some cases these spawnings have been normal. In others few eggs are released, subsequent development is often abnormal, and survival of the larvae is poor.

In our experiments, only *T. squamosa* released viable eggs. The two spawnings were spontaneous. All artificial methods induced only the release of sperm and some immature eggs. These results and those of others (see Jameson, 1976) suggest that regardless of the method of induction, the gonads must be mature before normal release of both eggs and sperm can occur.

In the tridacnids, the factors influencing gonad development and the initiation of normal spawning behavior *in situ* are not known. Seasonality, temperature, phases of the moon (tides), and water motion have all been implicated. Of these, a strong case can be made only for seasonality. It appears from our study, and others (see Yamaguchi, 1977), that tridacnid species have different breeding seasons (see Table II). This implies that in nature, different environmental cues may trigger gonad maturation and spawning. Our results indicate that water temperature is probably not a critical factor for the induction of spawning of tridacnids *in situ* (also see Wada, 1954; LaBarbera, 1975).

The evidence concerning the importance of phases of the moon, time of day, tides, and currents to spawning is conflicting (LaBarbera, 1975; Jameson, 1976, Beckvar, 1981). Although spawning may be seasonal for a given species, the actual time span of spawning may vary from one year to another and may occur for only a brief period (Stephenson, 1934; Jameson, 1976; this study). Until factors influencing gonad development are determined, successful induction of normal spawning of eggs and sperm for experimental work or mariculture will depend on the investigator being in the right place at the right time.

Acquisition of zooxanthellae

Many corals, anemones, and hydroids pass their symbiotic algae directly to their offspring in sexual reproduction (Duerden, 1902; Mangan, 1909; Fraser, 1931; Abe, 1937; Kawaguti, 1940; Atoda, 1947a, b, 1951b, c, 1954; Harrigan, 1972). Yonge (1936) originally proposed maternal inheritance of zooxanthellae by tridacnids. However, Stephenson (1934) and Mansour (1946c) found no zooxanthellae in unreleased eggs in *H. hippopus* and *T. maxima* respectively. Our observations corroborate those of LaBarbera (1975) and Jameson (1976) in finding no zooxanthellae in spawned eggs. Each generation must acquire their symbionts from the environment. This phenomenon occurs in a surprising assortment of invertebrates, including the jellyfishes *Cassiopeia andromeda* (Gohar and Eisawy, 1960; Ludwig, 1969; Rahat and Adar, 1980), *Mastigias papua* (Sugiura, 1963, 1964), the gorgonians *Pseudopterogorgia bipinnata, P. elisabethae, Briareum asbestinum* (Kinzie, 1974), the corals *Astrangia danae* (Szmant-Froelich *et al.*, 1980), *Acropora bruggemanni* (Atoda, 1951a) and *Pocillopora meandrina* (Stimson, pers. com.), the anemones *Anthopleura elegantissima* and *A. xanthogrammica* (Siebert, 1974) and *A. tagetes* (G. Muller Parker, pers. com.), and the heart shell *Corculum cardissa* (Kawaguti, 1950).

Uptake and persistance of zooxanthellae in gastrodermal cells of coelenterates involves possible discrimination of strains on surface contact, during phagocytosis, and/or intracellular recognition after phagocytosis (Trench, 1981; Trench *et al.*, 1981). Our results with *T. squamosa* indicate that initial uptake of zooxanthellae is through the mouth and into the stomach, and is nondiscriminatory. Many zooxanthellae are subsequently seen in the viscera. After metamorphosis, zooxanthellae apparently pass from the alimentary system by an unknown mechanism into the developing siphonal tissues, where it often difficult to distinguish by electron micrographs whether they are intra- or extracellular. We interpret this stage as that which indicates the establishment of an association. Clams were able to form an association in this manner with any strain of *S. microadriaticum* introduced after the development of the larval esophagus, stomach, and intestine, These findings imply that in nature, individuals of the same species might not necessarily all contain the same strain of zooxanthellae, unless there is subsequent sorting and elimination of all but one strain, as is seen in reinfection studies on the flatworms *Convoluta roscoffensis* and *Amphiscolops langerhansi* (Provosoli *et al.*, 1968; Taylor, 1971b, 1980).

Tridacnids live in an environment where a number of amphidinioid and gymnodinioid zooxanthellae are potentially available. All reef-building corals and many gorgonians, sea anemones, and jellyfishes harbor *S. microadriaticum* in their tissues (Freudenthal, 1962; Taylor, 1969, 1973, 1974). Under certain conditions zooxanthellae are released from these hosts, often in a healthy vegetative or motile state (Mansour, 1946c; Goreau, 1964; Trench, 1974; Steele, 1975; Ricard and Salvat, 1977; Jaap, 1979; Trench *et al.*, 1981). These algae may be taken into the stomachs of larval, juvenile, or adult tridacnids (Fankboner and Reid, 1981).

When zooxanthellae are outside their hosts, either in seawater or culture media, they alternate between a coccoid non-motile form and a dumbell-shaped motile form (McLaughlin and Zahl, 1957, 1959, 1966; Freudenthal, 1962; Taylor, 1973; Loeblich and Sherley, 1979; Deane *et al.*, 1979). Previous studies on cultured zooxanthellae have shown that motile zooxanthellae are found only in the light phase of a normal light:dark cycle (Fitt *et al.*, 1981). Ricard and Salvat (1977) made similar observations on motile zooxanthellae in fecal pellets from freshly collected *T. maxima.* Motile zooxanthellae are thought to be important in the infection of potential aposymbiotic hosts (Kinzie, 1974; Steele, 1977; Trench, 1979, 1980). We have shown here that veligers are more likely to take up motile zooxanthellae than non-motile zooxanthellae. Veligers, being planktonic filter feeders, are more likely to encounter swimming rather than sessile algae. The ability of the veligers to take in motile zooxanthellae may be responsible for our differential survival results (Fig. 5, Table III). By this reasoning, the more motile zooxanthellae available, the more will be taken into the gut, with the possible result of more nutrition, leading to higher survival rates. *If* this is the case, intrinsic motility patterns of different strains of zooxanthellae (Fitt *et al.*, 1981) may be very important to the acquisition, nutrition, and survival of juvenile clams in nature. Strains of zooxanthellae which are motile throughout the day are more likely to be taken up (in greater quantities) than strains motile for only half of the day. Unfortunately, the natural diets of larval, juvenile, and adult tridacnids are not known, making it difficult to determine the relative importance of zooxanthellae to their nutrition. Jameson (1976) found that veligers of *H. hippopus, T. maxima*, and *T. crocea* all "fed" primarily on 5 μm flagellated cells when maintained in unfiltered seawater. These cells were not further identified.

Biological specificity in symbiotic associations has been addressed by Weiss (1953), Dubos and Kessler (1963), Schoenberg and Trench (1980c), and Trench *et al.*, (1981). One may consider two levels of specificity in the association between *S. microadriaticum* and the Tridacnidae. The first level concerns the species of algae that live endosymbiotically in tridacnids. Although many species of phytoplankton inhabit coral reef waters, including at least one other symbiotic dinoflagellate (*Amphidinium* sp.), only the dinoflagellate *S. microadriaticum* is found in the tissues of tridacnids. In addition, no adult clams are found without this species of alga. These facts alone imply the existence of a selection process that enables *S. microadriaticum* to establish and maintain a stable relation with its host. Our data suggest possible mechanisms by which selection may take place.

A second level of specificity involves strains of *S. microadriaticum* in tridacnid clams. It is not clear whether there is a selective advantage for clams having a particular strain of *S. microadriaticum*, as suggested in marine coelenterate symbiosis (Schoenberg and Trench, 1980c; Kinzie and Chee, 1979). We found no significant difference in sizes of veligers with different strains of zooxanthellae. However, there was large variability in size and times of development of these veligers. Juveniles infected with different isolates of zooxanthellae demonstrated different growth rates. Kinzie and Chee (1979) found a similar phenomenon with different isolates of zooxanthellae injected into the anemone *Aiptasia pulchella.* Although not apparently important in growth and development of veligers, specific strains of zooxanthellae appear to be important to growth of juveniles and may also influence adult growth and reproductive success. Such is the case with *C. roscoffensis* and *A. langerhansi,* which may harbor many different species of algae, but only develop gonads and spawn with their naturally occurring symbiont (Provosoli *et al.,* 1968; Taylor, 1971b). The growth, survival, and reproductive success of adult tridacnids with different strains of zooxanthellae is not known.

These findings may have practical application. Tridacnid clams were once widespread thoughout the Indo-Pacific, but in the face of intense overfishing in many areas, these clams, particularly the larger members of the group, are facing extinction (Pearson, 1977). Several groups have initiated studies aimed at mariculture of "giant clams", mostly from the point of view of rearing them as a potential food source (Beckvar, 1981; Yamaguchi, 1977; Maclean, 1975). Knowledge of spawning and development of tridacnids, with particular reference to their association with zooxanthellae, may be important in future attempts at the mariculture of these animals.

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LITERATURE CITED

ABE, N. 1937. Post-larval development of the coral *Furgia actiniformis var. palawensis* Döderlien, *Palao Trop. Biol. Stat. Studies* **1**: 73–93.

AHLES, M. D. 1967. Some aspects of the morphology and physiology of *Symbiodinium microadriaticum*. Ph.D. dissertation. Fordham Univ., N. Y. 158 pp.

ATODA, K. 1947a. The larva and postlarval development of some reef-building corals. I. *Pocillopora damacornis cespitova* (Dana). *Sci. Rep. Tohoku Univ. Fourth Ser. (Biol.)* **18**: 24–47.

ATODA, K. 1947b. The larva and postlarval development of some reef-building corals. II. *Stylophora pistillata* (Esper.). *Sci. Rep. Tohoku Univ. Fourth Ser. (Biol.)* **18**: 48–64.

ATODA, K. 1951a. The larva and post larval development of the reef-building corals. III. *Acropora brüggemanni. J. Morphol.* **89**: 1–13.

ATODA, K. 1951b. The larva and post larval development of the reef-building corals. IV. *Galaxea aspera* Quelch. *J. Morphol.* **89**: 17–30.

ATODA, K. 1951c. The larva and post larval development of some reef-building corals. V. *Seriatopora hystrix.* Dana. *Sci. Rep. Tôhoku Univ. Fourth Ser. (Biol.)* **19**: 33–39.

ATODA, K. 1954. Post larval development of the sea anemone, *Anthopleura* sp. *Sci. Rep. Tôhoku Univ. Fourth Ser. (Biol.)* **20**: 274–288.

BECKVAR, N. 1981. Cultivation, spawning, and growth of the giant clams *Tridacna gigas, T. derasa,* and *T. squamosa* in Palau, Caroline Islands. Aquaculture **24**: 21–30.

BOSCHMA, H. 1924. On the food of madreporaria. *Proc. Kon. Ak. Wet. Amsterdam.* **27**: 13–23.

BROCK, J. 1888. Über die sogenannten Augen von *Tridacna* and das Verkommen von Pseudochlorophyll körporen im Gëfussystem der Muscheln. *Z. Wiss. Zool.* **66**: 270–288.

COE, W. R. 1948. Nutrition, environmental conditions, and growth of marine bivalve mollusks. *J. Mar. Res.* **7**: 586–601.

DEAN, D. 1958. New property of the crystalline style of *Crassostrea virginica. Science* **128**: 837.

DEANE, E. M., T. DUXBURY, AND R. W. O'BRIEN. 1979. Growth cycle of free-living *Gymnodinium microadriaticum* from *Tridacna maxima. Br. Phycol. J.* **14**: 407–412.

DUBOS, R., AND A. KESSLER. 1963. Integrative and disintegrative factors in symbiotic associations. *Symp. Soc. Gen. Microbiol.* **13**: 1–11.

DUERDEN, J. E. 1902. West Indian madreporarian polyps. *Nat. Acad. Sci. Wash.* **8**, 7th Memoir: **403**: 597.

EDSON, K. M., S. B. VINSON, D. B. STOLZ, AND M. D. SUMMERSL. 1981. Virus in a parasitoid wasp: suppression of the cellular immune response in the parasitoid's host. *Science* **211**: 582–583.

FANKBONER, PETER V. 1971. Intracellular digestion of symbiotic zooxanthellae by host amoebocytes in giant clams (Bivalvia: Tridacnidae), with a note on the nutritional role of the hypertrophied siphonal epidermis. *Biol. Bull.* **141**: 222–234.

FANKBONER, P. V., AND R. E. REID. 1981. Mass expulsion of zooxanthellae by heat-stressed corals: a source of food for giant clams? *Experimentia* **37**: 251–252.

FITT, W. K., S. S. CHANG, AND R. R. TRENCH. 1981. Motility patterns of different strains of the symbiotic dinoflagellate *Symbiodinium (= Gymnodinium) microadriaticum* (Freudenthal) in culture. *Bull. Mar. Sci.* **31**: 436–443.

FRASER, E. A. 1931. Observations on the life-history and development of the hydroid *Myrionema amboinense. Sci. Repts. Great Barrier Reef Exp.* **3:** 135–144.

FREUDENTHAL, H. D. 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: taxonomy, life cycle, and morphology. *J. Protozool.* **9:** 45–52.

GOHAR, H. A. F., AND A. M. EISAWY. 1960. The development of *Cassiopea andromeda. Publ. Mar. Biol. Sta. Al-Ghardaga Egypt* **11:** 148–190.

GOREAU, T. F. 1964. Mass expulsion of zooxanthellae from Jamaican reef communities after Hurricane Flora. *Science* **145:** 383–386.

GOREAU, T. F., N. I. GOREAU, AND C. M. YONGE. 1973. On the utilization of photosynthetic products from zooxanthellae and of a dissolved amino acid in *Tridacna maxima* f. *elongata* (Mollusca:Bivalvia). *J. Zool. Lond.* **169:** 417–454.

HARDY, J. T., AND S. A. HARDY. 1969. Ecology of *Tridacna* in Palau. *Pacific Sci.* **23:** 467–472.

HARRIGAN, J. 1972. The planula larva of *Pocillopora damicornis;* lunar periodicity of swarming and substratum selection behavior. Ph. D. Thesis. Univ. of Hawaii, Honolulu. 313 p.

HAVEN, D. S. AND R. MORALES-ALAMO. 1966. Aspects of biodeposition of oysters and other invertebrate filter feeders. *Limnol. Ocean.* **11:** 487–498.

HILDRETH, D. I. 1980. The passage of two species of micro-algae through the gut of *Mytilus edulus* L. *J. Exp. Mar. Biol. Ecol.* **48:** 17–22.

JAAP, W. C. 1979. Observations on zooxanthellae expulsion at Middle Sambo Reef, Florida Keys. *Bull. Mar. Sci.* **29:** 414–422.

JAMESON, S. C. 1976. Early life history of the giant clams *Tridacna crocea* Lamarck, *Tridacna maxima* (Röding) and *Hippodus hippopus* (Linnaeus). *Pacific Sci.* **30:** 219–233.

KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27:** 137–138A.

KAWAGUTI, S. 1940. On the physiology of reef corals. V. Tropism of coral planulae, considered as a factor of distribution of the reefs. *Palao Trop. Biol. Stat. Studies* **2:** 319–328.

KAWAGUTI, S. 1950. Observations of the heart shell, *Corculum cardissa* (L.), and its associated zooxanthellae. *Pacific Sci.* **4:** 43–49.

KAWAGUTI, S. 1968. Electron microscopy on zooxanthellae in the mantle and gill of the heart shell. *Biol. J. Okayama University* **14:** 1–12.

KINZIE, R. A. 1974. Experimental infection of aposymbiotic gorgonian polyps with zooxanthellae. *J. Exp. Mar. Biol. Ecol.* **15:** 335–345.

KINZIE, R. A., III, AND G. S. CHEE. 1979. The effect of different zooxanthellae on the growth of experimentally reinfected hosts. *Biol. Bull.* **156:** 315–327.

LABARBERA, M. 1975. Larval and post larval developments of the giant clams *Tridacna maxima* and *Tridacna squamosa* (Bivalvia:Tridacnidae). *Malacologia* **15:** 69–79.

LOEBLICH, A. R., III, AND J. L. SHERLEY. 1979. Observations on the theca of the motile phase of free-living and symbiotic isolates of *Zooxanthella microadriatica* (Freudenthal) Comb. nov. *J. Mar. Biol. Assoc. U. K.* **59:** 195–205.

LOOSANOFF, U. L., AND H. C. DAVIS. 1963. Rearing of bivalve mollusks. pp. 1–136 in F. S. Russel, Ed., *Advances in Marine Biology,* Vol. 1, Academic Press, N. Y.

LUDWIG, L. D. 1969. Die zooxanthellen bei *Cassiopea andromeda* Eschscholtz 1829 (Polyp-Stadium) und ihre Bedeutung für die strobilation. *Zool. Jb. Anat. Bd.* **86:** 238–277.

MACIEAN, J. L. 1975. *The potential of Aquaculture in Australia.* Australian Govt. Publ. Service, Canberra.

MANGAN, J. 1909. The entry of zooxanthellae into the ovum of *Millepora,* and some particulars concerning the medusae. *Q. J. Microsc. Sci.* **33:** 697–709.

MANSOUR, K. 1946a. Source and fate of the zooxanthellae of the visceral mass of *Tridachna elongata. Nature* **158:** 130.

MANSOUR, K. 1946b. Communication between the dorsal edge of the mantle and the stomach of *Tridacna. Nature* **157:** 844.

MANSOUR, K. 1946c. The zooxanthellae, morphological pecularities and food and feeding habits of the Tridacnidae with reference to other lamellibranchs. *Egyptian Acad. Sci. Proc.* **1:** 1–11.

MCLAUGHLIN, J. J. A., AND P. A. ZAHL. 1957. Studies in marine biology. II. *In vitro* culture of zooxanthellae. *Proc. Soc. Exptl. Med.* **95:** 115–120.

MCLAUGHLIN, J. J. A., AND P. A. ZAHL. 1959. Axenic zooxanthellae from various hosts. *Ann. N. Y. Acad. Sci.* **77:** 55–72.

MCLAUGHLIN, J. J. A., AND P. A. ZAHL. Endozoic Algae. Pp. 257–297 in S. M. Henry, Ed., *Symbiosis,* Vol. 1. Academic Press, N. Y.

MORSE, D. E., H. DUNCAN, N. HOOKER, AND A. MORSE. 1977. Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. *Science* **196:** 298–300.

MORTON, B. 1978. The diurnal rhythm and the processes of feeding and digestion in *Tridacna crocea* (Bivalve:Tridacnidae). *J. Zool. Cond.* **185:** 371–387.

MUSCATINE, L. 1973. Nutrition of corals. Pp. 77–115 in O. A. Jones and R. Endean, Eds. *Biology and Geology of Coral Reefs.* Vol. II. Academic Press, N. Y.

PEARSON, R. G. 1977. Impact of foreign vessels poaching giant clams. *Austrialian Fisheries* **7:** 8–23.

PROVASOLI, L., T. YAMASU, AND I. MANTON. 1968. Experiments on the resynthesis of symbiosis in *Convoluta roscoffensis* with different flagellate cultures. *J. Mar. Biol. Assoc. U. K.* **48:** 465–479.

RAHAT, M., AND O. ADAR. 1980. Effect of symbiotic zooxanthellae and temperature on budding and strobilation in *Cassiopeia andromeda* (Eschscholz). *Biol. Bull.* **159:** 394–401.

RICARD, M., AND B. SALVAT. 1977. Faeces of *Tridacna maxima* (Mollusca:Bivalvia) composition and coral reef importance. *Proc. Third Coral Reef Symp.,* 495–501.

ROSEWATER, J. 1965. The family Tridacnidae in the Indo Pacific. *Indo-Pacific Mollusca* **1:** 347–396.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980a. Genetic variation in *Symbiodinium (= Gymnodinium) microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. I. Isoenzyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum. Proc. R. Soc. Lond. B. Biol. Sci.* **207:** 405–427.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980b. Genetic variation in *Symbidinium (= Gymnodinium) microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. II. Morphological variation in *Symbiodinium microadriaticum. Proc. R. Soc. Lond. B. Biol. Sci.* **207:** 429–444.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980c. Genetic variation in *Symbiodinium (= Gymnodinium) microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. III. Specificity and infectivity of *Symbiodinium microadriaticum. Proc. R. Soc. Lond. B. Biol. Sci.* **207:** 445–460.

SIEBERT, A. E. 1974. A description of the embryology, larval development, and feeding of the sea anemone *Anthopleura elegantissima* and *A. xanthogramica. Can. J. Zool.* **52:** 1383–88.

STEELE, R. D. 1975. Stages in the life history of a symbiotic zooxanthella in pellets extruded by its host *Aptasia tagetes* (Duch. and Mich.) (Coelenterata, Anthozoa). *Biol. Bull.* **149:** 590–600.

STEELE, R. D. 1977. The significance of zooxanthella-containing pellets extruded by sea anemones. *Bull. Mar. Sci.* **27:** 591–594.

STEPHENSON, A. 1934. The breeding of reef animals. II. Invertebrates other than coral. *Sci. Rpts. Gr. Barrier Reef Exped.* **3:** 247–272.

SUGIURA, Y. 1963. On the life-history of rhizostome medusae. I. *Mastigias papua* L. Agassiz. *Annot. Zool. Jpn.* **36:** 194–202.

SUGIURA, Y. 1964. On the life-history of rhizostone medusae. II. Indispensibility of zooxanthellae for strobilation in *Mastigias papua. Embryologica* **8:** 223–233.

SZMANT-FROELICH, A., P. YEUICH, AND M. E. Q. PILSON. 1980. Gametogenesis and early development of the temperate coral *Astrangia danae* (Anthozoa: Scleractinia). *Biol. Bull.* **158:** 257–269.

TAYLOR, D. L. 1969. Identity of zooxanthellae isolated from some Pacific Tridacnidae. *J. Phycol.* **5:** 336–340.

TAYLOR, D. L. 1971a. Ultrastructure of the "zooxanthella" *Endodinium chattonii* in situ. *J. Mar. Biol. Assoc. U. K.* **51:** 227–234.

TAYLOR, D. L. 1971b. On the symbiosis between *Amphidinium klebsii* (Dinophyceae) and *Amphiscolops langerhansi* (Turbellaria:Acoela). *J. Mar. Biol. Assoc. U. K.* **51:** 301–313.

TAYLOR, D. L. 1973. Algal symbionts of invertebrates. *Ann. Rev. Microbiol.* **27:** 171–187.

TAYLOR, D. L. 1974. Symbiotic marine algae: taxonomy and biological fitness. Pp. 245–262 in W. B. Vernberg, Ed., *Symbiosis in the Sea.* Univ. South Carolina Press, Columbia, S.C.

TAYLOR, D. L. 1980. Nutrient competition as a basis for symbiont selection in associations involving *Convoluta roscoffensis* and *Amphiscolops langerhansi.* Pp. 279–291 W. Schwemmler and H. E. A. Schenk, Eds., *Endocytobiology,* Vol. 1. Walter de Gruyter and Co., Berlin, N. Y.

TRENCH, R. K. 1974. Nutritional potentials in *Zoanthus sociatus* (Coelenterata, Anthozoa). *Helgolander Wiss. Meersunters* **26:** 174–216.

TRENCH, R. K. 1979. The cell biology of plant-animal symbiosis. *Ann. Rev. Plant Physiol.* **30:** 485–532.

TRENCH, R. K. 1980. Integrative mechanisms in mutualistic symbioses. Pp. 275–297 Eds. C. B. Cook, P. W. Pappas, and E. D. Rudolf, Eds. *Cellular Interactions in Symbiosis and Parasitism.* Ohio State Univ. Press, Columbus. p. 275–97.

TRENCH, R. K. 1981. Cellular and molecular interactions in symbioses between dinoflagellates and marine invertebrates. *Pure and Applied Chemistry.* **53:** 819–835.

TRENCH, R. K, N. J. COLLEY, AND W. K. FITT. 1981. Recognition phenomena in symbioses between marine invertebrates and "zooxanthellae"; uptake, sequestration and persistence. *Ber. Beuts. Bot. Gesellshaft.* **94:** 568–577.

Trench, R. K., D. S. Wethey, and J. W. Porter. 1981. Some observation on the symbiosis with zooxanthellae among the tridacnidae (Mollusca, Bivalvia). *Biol. Bull.* **161:** 180–198.

Wada, S. K. 1952. Protandric functional hermaphroditism in the Tridacnid clams. *Oceanographical Magazine Tokyo* **4:** 23–30.

Wada, S. K. 1954. Spawning in the tridacnid clams. *Jpn. J. Zool.* **11:** 273–285.

Weiss, P. 1953. Specificity in growth control. Pp. 195–206 *Biological Specificity and Control.* E. G. Butleer, Ed., Princeton Univ. Press, Princeton, N. J.

Yamaguchi, M. 1977. Conservation and cultivation of giant clams in the tropical Pacific. *Biol. Conserv.* **11:** 13–20.

Yonge, C. M. 1936. Mode of life, feeding, digestion and symbiosis with zooxanthellae in the Tridacnidae. *Sci. Repts. Gr. Barrier Reef Exped.* **1:** 283–321.

Yonge, C. M. 1975. Giant clams. *Sci. Am.* **232:** 96–105.

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HISTOLOGY OF A BILATERAL GYNANDROMORPH OF THE BLUE CRAB, *CALLINECTES SAPIDUS* RATHBUN (DECAPODA: PORTUNIDAE)

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ABSTRACT

The anatomy and histology of a gynandromorphic blue crab, *Callinectes sapidus*, are described. This is the first bilateral gynandromorph reported in a species of the Brachyura. Externally there was perfect bilateral division into male and female sides. The gonad on the male side was morphologically and histologically normal, but an AVD (anterior vas deferens) had been differentiated on the female side, and spermatogenesis was occurring through much of the female gonad. However, oogenesis was taking place in the apical portions of the gonadal lobes and a normal seminal receptacle was present. The crab had copulated as a female and may have copulated as a male.

Function of the malacostracan AG (androgenic gland) in control of maleness is discussed. It is concluded that sexual differentiation in malacostracan species exhibiting bilateral gynandromorphism must depend on complex mechanisms. External sexual characters may be determined very early in embryogenesis, but differentiation of the gonads is apparently influenced by the AG and other parts of the hormonal systems.

INTRODUCTION

A blue crab, *Callinectes sapidus* Rathbun, that displayed perfect external bilateral gynandromorphism, was taken from a tributary of Chesapeake Bay. It has been briefly described by Otto (1979). This apparently is the only blue-crab gynandromorph that has been brought to the attention of scientists concerned with the species, and is the only bilateral gynandromorph reported in the Brachyura. Considering that blue crabs have been fished commercially for many years, especially in the Chesapeake Bay region, and that male and female blue crabs are easily distinguished externally, one must conclude that bilateral gynandromorphism is extremely rare in this decapod species. The rarity of externally evident gynandromorphism of any kind in dioecious (nonhermaphroditic; gonochoristic) decapods is further demonstrated by the facts that Hartnoll (1960) found only one gynandromorphic *Hyas coarctatus* of 2500 examined, George (1963) reported one gynandromorph among 16,870 specimens of *Metapenaeus monoceros,* and Farmer (1972) discovered but one gynandromorph in the 40,000 specimens of *Nephrops norvegicus* he examined.

Functional and nonfunctional hermaphroditism occurs in several groups of malacostracan crustaceans, but gynandromorphism apparently is confined to the Decapoda. Farmer (1972) and Charniaux-Cotton (1975) believe that bilateral gyn-

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Abbreviations: AG, androgenic gland; AVD, anterior vas deferens; MVD, median vas deferens; PVD, posterior vas deferens.

andromorphism in decapods is of genetic origin. It could be due to loss of a chromosome during early embryogenic cleavage. If the initial embryonic cleavage were determinate, a single abnormal division involving sex chromosomes would be sufficient to cause the condition.

Partial gynandromorphism has been observed in species of macrurans, anomurans, and brachyurans (see Hartnoll, 1960, and Charniaux-Cotton, 1975), but the literature mentions occurrence of bilateral gynandromorphs only in species of macrurans. Based on the literature and his own investigations, Farmer (1972) listed the following cases: two specimens of *Homarus gammarus,* two of *Homarus americanus,* two of *Nephrops norvegicus,* and one of *Palinurus frontalis.* The internal anatomy was investigated in one specimen each of *N. norvegicus, H. gammarus,* and *H. americanus.* In these three animals, division of the gonads into testis-vas deferens and ovary was also bilateral and corresponded to the external condition. The one gynandromorphic *N. norvegicus* examined histologically had mature sperm in the testis and large oocytes in the ovary. Both testis and ovary appeared normal (Farmer, 1972). One gynandromorphic *H. americanus* was also examined histologically. This lobster had mature sperm in the testis and vas deferens; the ovary contained maturing ova and some possibly degenerate ones (Chace and Moore, 1959). Neither Farmer (1972) nor Chace and Moore (1959) mentioned whether vitellogenesis was occurring in their specimens, but because they speak of large and maturing ova, presumably it was. The AG (androgenic gland) was not searched for in either specimen.

It is believed that all malacostracan crustaceans have AGs. Expression of primary and secondary sexual characters of the male is controlled by the AG in species of amphipods, isopods, and protandric hermaphroditic natantian decapods that have been studied. If young females receive AG implants, the ovaries transform into testes and external male characteristics develop with successive molts. Conversely, males deprived of AGs develop ovaries and female secondary characters (Charniaux-Cotton, 1975). Because early experiments with AG implants and ablations in dioecious brachyuran decapods were only marginally successful in modifying sexual characters, it was thought that expression of sex in dioecious decapods might differ from its expression in amphipods, isopods, and protandric decapods (Payen, 1969; Charniaux-Cotton, 1975). Recently, however, females of two dioecious species of natantians were masculinized by AG implants. Partial masculinization was obtained in *Palaemonetes varians* (Charniaux-Cotton and Cazes, 1979) and complete masculinization in *Macrobrachium rosenbergii* (Nagamine *et al.,* 1980b). Nagamine *et al.* (1980a) also induced feminization of young *M. rosenbergii* males by ablating the AGs.

The bilateral gynandromorph of the blue crab offered an opportunity to investigate histologically the condition of the gonads and other organs in a dioecious brachyuran whose external secondary sexual characters were unequivocally male on one side and female on the other, and to assess the histological data in the light of the recent results of AG implantation and ablation in the dioecious natantian decapods mentioned above.

MATERIALS AND METHODS

The gynandromorphic blue crab was taken on 10 July 1979 in a commercial crab trap set in Broad Creek (a tributary of Chesapeake Bay), Talbot County, Maryland. It was maintained out of water for the remainder of that day and transported on ice to the Oxford Laboratory the following morning. It was moribund when received and dead when it was dissected in the early afternoon. The owner of the crab had requested that the ventral and lateral parts not be damaged during dissection. Therefore, only the median part of the carapace was removed, and neither the AGs nor the Y organs (molting glands) could be taken.

On the male side of the crab, samples were taken of the AVD (anterior vas deferens), MVD (median vas deferens), PVD (posterior vas deferens), and of the testis, both proximal to the AVD and as near the anterior tip of the testis as possible. On the female side, four samples were taken of the gonad; the first was from near the AVD, the second from the median area of the anterior lobe, the third from the apical part of the anterior lobe, as near the tip as possible, and the fourth from the most distal part of the posterior lobe. The entire seminal receptacle was taken. Other tissues and organs sampled included the entire brain and thoracic ganglion, and parts of the hepatopancreas, midgut, midgut ceca, hemopoietic tissue, antennal gland, epidermis, gill, mandibular organ, and heart (see Johnson, 1980).

The tissues were fixed in Helly's fluid, dehydrated, and embedded in paraffin in the usual manner. Tissue sections were stained with hematoxylin-eosin, with alcian blue-nuclear fast red, and by the periodic acid-Schiff and Feulgen techniques (Johnson, 1980).

Results

External anatomy

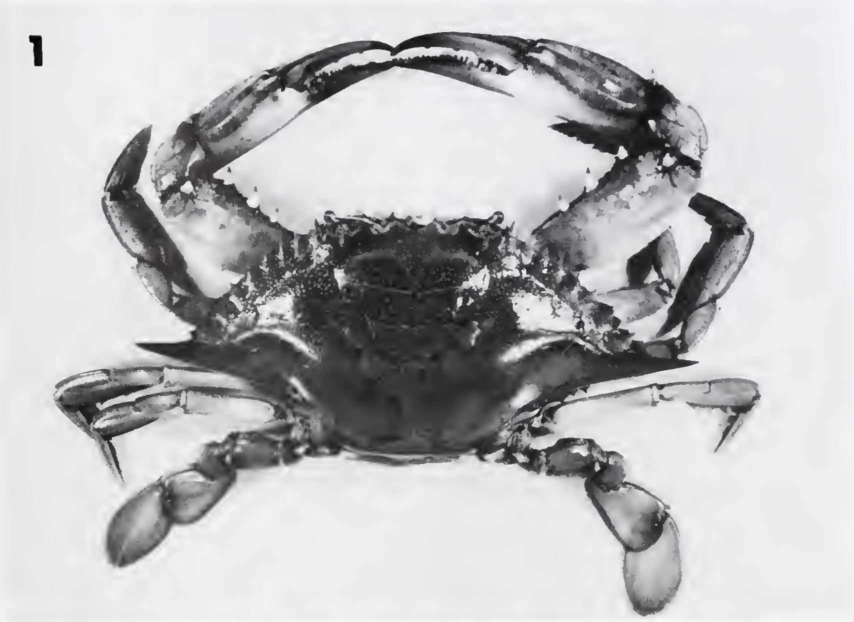
The crab was large (18 cm carapace width) and in good condition. The exoskeleton was hard, but free of blemishes, indicating that the animal had molted relatively recently. External sexually dimorphic characters were male on the right side and female on the left side (Figs. 1, 2). The male cheliped was blue and larger than the female cheliped, which was red tipped, as is typical of female blue crabs. The anterolateral margin of the carapace was less convex on the male side. The male half of the abdomen was narrow; the female half was broad, with the convex lateral margin of a mature female (Fig. 2). The pleopods had the male form on the right and female form on the left (Fig. 3).

Internal anatomy

All the internal organs, except the gonads, were of normal appearance. As in normal blue crabs, the gonads formed an "H," with an anterior and a posterior lobe on each side and a central crossbridge posterior to the stomach.

On the male side, a slender testis extended anterolaterally as far as it could be followed. The coiled white AVD occupied its normal position close to the median bridge, and was of the usual size. When it was cut, numerous spermatophores escaped from its lumen. The MVD was also in its usual position, being somewhat lateral to the AVD, and partly obscured by it. It was pinkish and much smaller than would be expected in a normal male that had not copulated recently. The PVD was of the normal greenish, translucent color and ran posteriorly in the ventral part of the body cavity. Unlike the PVD of a normal mature male that has not copulated recently, it was not bulbous and distended with secretion, but was tubular and with a variable diameter that did not greatly exceed the diameter of the midgut in most parts.

On the female side, the anterior lobe of the gonad was white and about the same diameter as the testis. Proximal to the crossbridge of the gonad was a white,



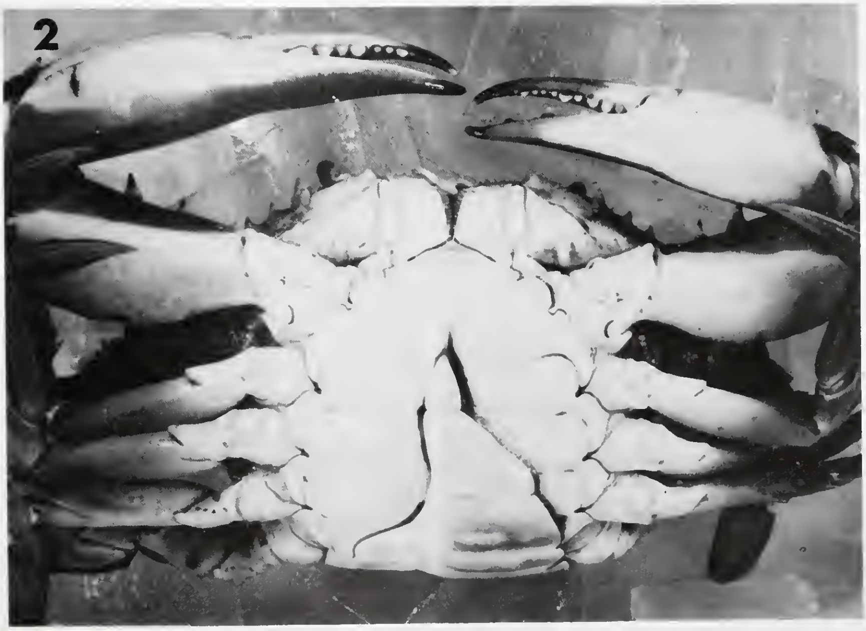


FIGURE 1. Dorsal view of the gynandromorph; male to right, female to left.
FIGURE 2. Ventral view of the gynandromorph; male to left, female to right.

coiled, tubular part that appeared like, and occupied the usual position of the AVD. It was a functioning AVD, as demonstrated by the release of spermatophores when it was cut. There was no sign of an MVD or a PVD. The posterior lobe of the gonad was very slender, white, and difficult to follow. It did not differ in appearance from the posterior ovarian lobe of an immature female, or the testicular lobe of a young male. Like the posterior lobe of the normal ovary, it was dorsal and ended posteriorly in the region of the posterior midgut cecum. The seminal receptacle of the blue crab is white, except for a variable period following copulation. At that time, it is enlarged and pink, due to presence of the pink sperm plug. The seminal receptacle of the gynandromorph was small and flat, but it was pinkish, indicating retention of a part of the sperm plug.

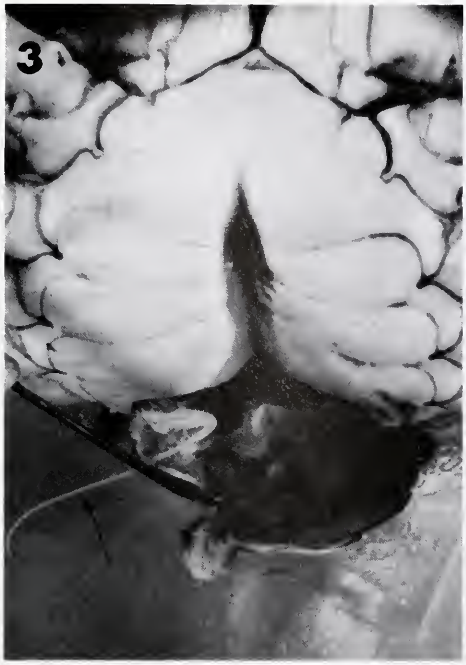
Histological appearance

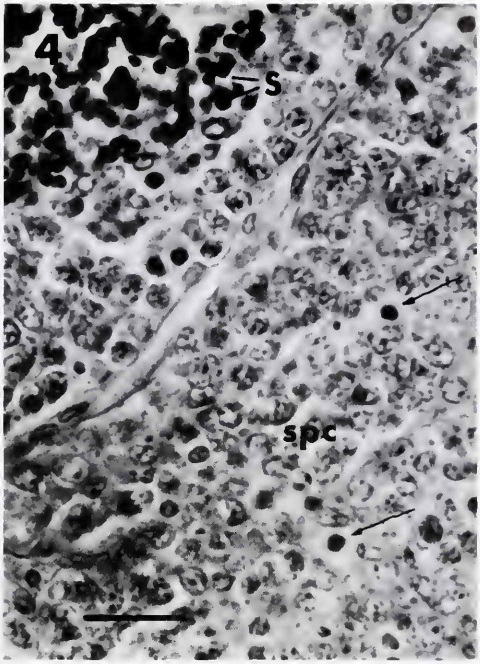
The internal organs and tissues appeared normal except for the gonads. The crab was in intermolt (stage C). Neurosecretory cells of the brain and thoracic ganglion were morphologically similar on the two sides. The crab was well nourished. There was considerable glycogen in the spongy connective tissues; and the reserve-inclusion cells, that store hemocyanin and other materials and are associated with the connective tissues and hepatopancreas, were normal in size and number. The hepatopancreatic tubules and the antennal gland were autolysing. Other tissues showed little or no degeneration, except that the midgut epithelium had fragmented and sloughed into the lumen, and mitosis in the hemopoietic tissue was arrested in prophase. Most of the prophase nuclei were pyknotic. There was no evidence of microbial or parasitic infection except for three degenerate gregarines in the lumen of the posterior midgut cecum.

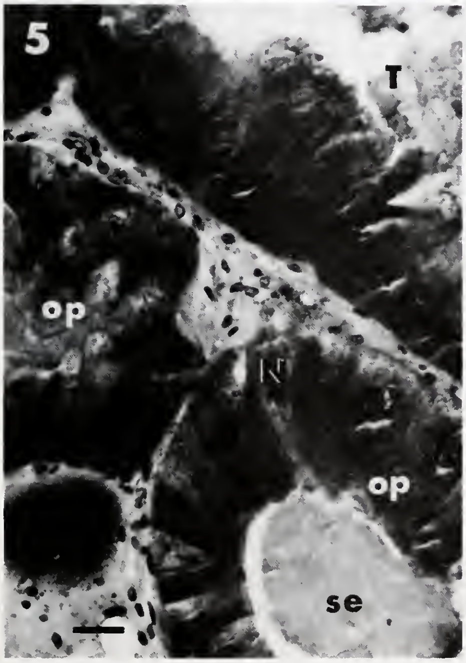
Testicular lobules on the male side contained primary and secondary spermatocytes, spermatids, and developing sperm. All the cells in a single lobule were in one developmental stage, as typical of mature blue-crab males. Few gonia (stem cells) were visible in the sections, and mitosis was rare. Some primary spermatocytes were in prophase, but the nuclei appeared to be pyknotic (Fig. 4). There were many mature sperm in the seminiferous duct.

The AVD had a normal, thick epithelium. Both forming and fully encapsulated spermatophores were present in the lumen, surrounded by the usual heterogeneous secretion. Epithelia of the MVD and PVD had the large, lobed nuclei typical of mature males. Lumina of the MVD and PVD contained secretions typical of the particular part of the vas deferens, but neither part was greatly distended with secretion, and outpocketings from the main lumen were evident (Fig. 5).

On the female side, adjacent to the crossbridge and the abnormally present AVD, the gonadal lobules contained primary spermatocytes (Fig. 6), and just distal to this portion, lobules were filled with primary spermatocytes, spermatids, and developing sperm. As on the male side, each lobule contained cells in a single stage of development. Proximal to the AVD, the seminiferous duct was thick walled (Fig. 6), and resembled the normal male seminiferous duct near its entrance into the AVD. In the median part of the anterior lobe of the gonad, most lobules contained secondary spermatocytes. There were few gonia and no spermatids or developing sperm in sections of the median sample, but the seminiferous duct here was full of mature sperm. In most cross sections, the seminiferous duct had a columnar epithelium around the entire circumference, rather than having one side opening broadly into a lobule, as it does in normal testis. The distal part of the anterior lobe of the gonad contained oocytes and a few gonia. The oocytes were like those of females just before and after the pubertal molt. Many of the larger ones contained "yolk nuclei," which are considered indicators of impending vitellogenesis (Cronin, 1942; Johnson, 1980). There were fewer oocytes per lobule than normal, and they were broadly separated by cytoplasm that presumably belonged to the accessory







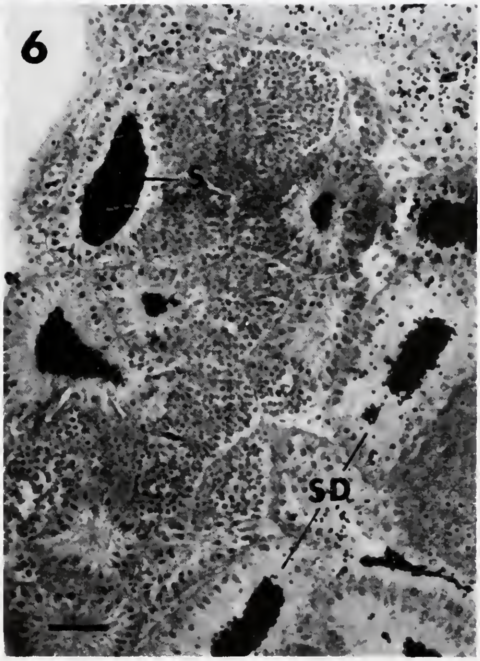
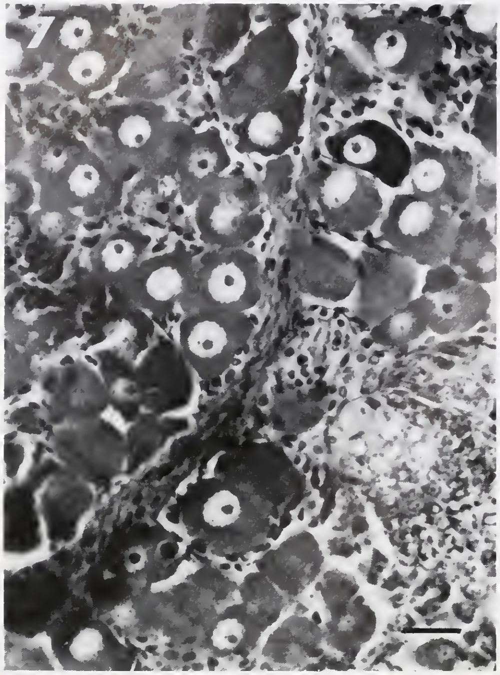


FIGURE 3. Venter of abdomen of the gynandromorph. Note long first pleopod on the male side (arrow).

FIGURE 4. Testicular lobules on the male side. Arrows point to arrested mitoses. Periodic acid-Schiff. spc = primary spermatocytes; S = sperm. Bar = 20 μm.

FIGURE 5. PVD on the male side. Hematoxylin and eosin. N = nucleus of PVD epithelium; op = outpocketing from main tubule; se = secretion in lumen; T = main tubule. Bar = 20 μm.

FIGURE 6. Testicular lobules on the female side, near the AVD. Lobules contain primary spermatocytes. Note the thick epithelium of the seminiferous duct (SD) and the sperm (S) within it. Periodic acid-Schiff. Bar = 50 μm.



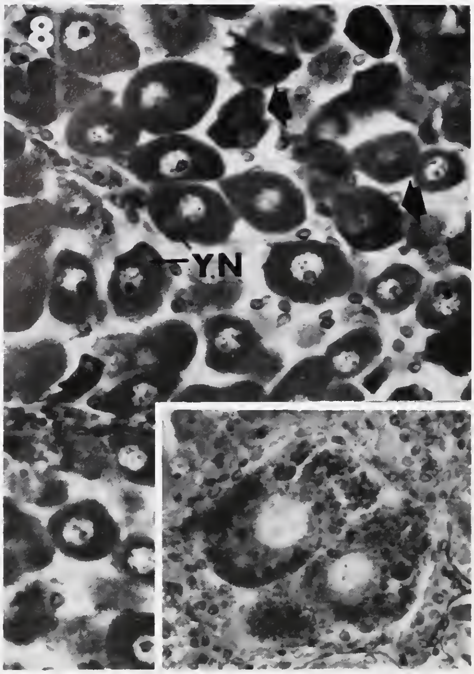


FIGURE 7. Ovarian lobules from a normal, molting, pubertal female. Periodic acid-Schiff. Bar = 20 μm.

FIGURE 8. Ovarian lobule from the posterior gonadal lobe on the female side. Arrows point to degenerating oocytes. Hematoxylin and eosin. Inset: vitellogenic oocytes from the anterior gonadal lobe on the female side. Periodic acid-Schiff. YN = yolk nucleus. Same scale as Figure 7.

(follicle) cells (compare Figs. 7 and 8). Degenerating oocytes were more common than in normal crabs. In one small area, several oocytes were undergoing early vitellogenesis (Fig. 8, inset). The apex of the posterior lobe of the gonad contained previtellogenic oocytes similar to those in the tip of the anterior lobe. Oocytes undergoing vitellogenesis were not seen in the posterior apex.

The seminal receptacle appeared like that of a mature female. The lumen was open and the more dorsal portions of the epithelium had sloughed into the lumen. A remnant of the sperm plug was present in the ventral part of the lumen, and a few sperm were associated with it.

DISCUSSION

It was not possible to search for the AG during dissection of the gynandromorph. If differentiation of male sexual characters of the blue crab is partially or wholly dependent on presence of the AG, one must have been present in the gynandromorph, at least until male puberty. Partial differentiation of the gonad on the female side into an AVD and a functioning testis indicates that the AG may have been functional at the time of death.

Ablation of the AG causes an inhibition of spermatogenesis in the dioecious natantians *Crangon* and *Leander,* and a hormone produced by the protocerebrum is necessary for maintenance of spermatogenesis (Touir, 1977a, b, c). Because there were large numbers of spermatophores and mature sperm in the testis and AVD of the blue-crab gynandromorph, lack of mitoses in its testicular lobules probably was not due to the possible lack of an AG or disturbance of protocerebral hormones, but because of the impaired physiological condition of the crab. It had been stressed by being out of water for at least 24 h, and had been kept at low temperatures for a part of that time. Cessation of mitosis in the hemopoietic tissue, and probable premortem termination of function in the hepatopancreas, midgut, and antennal gland also indicated physiological dysfunction.

Reasons for reduced secretion in the MVD and PVD were not readily evident. The condition of the epithelial nuclei, which were large and lobed, indicated that the MVD and PVD were in a fully secretory state. The crab may have recently copulated as a male. During copulation, secretion is expelled from the MVD and PVD and they contract, resulting in outpocketings from the main lumen like those in the gynandromorph (Johnson, 1980). However, the AVD of the gynandromorph contained many spermatophores. Usually, after copulation in a normal male, very few spermatophores remain in the AVD. The possibility exists that reduced secretion may have depended on interference with function of the AG or other parts of the male hormonal system, rather than on recent copulation.

The AG's influence on sexual behavior of crustaceans has not been studied. Sperm were in the seminal receptacle of the gynandromorph, and a portion of the sperm plug remained in the receptacle, showing that copulation with a male had occurred following the female pubertal molt. The male blue crab carries the prepubertal female during her premolt stage and copulates with her directly following molt. Except for prepubertal females, blue crabs exhibit agonistic behavior during premolt (Johnson, personal observation). For the crab to have copulated as a female, agonistic behavior regulated by the male side of the premolt gynandromorph would have had to have been overridden by the female behavior pattern. Few sperm were in the seminal receptacle, indicating that copulation had not been completely successful. Probably the presence of a male morphology on one side of the crab had interfered with the normal procedure.

Male hormones had influenced the remaining ovarian tissue on the female side. Early vitellogenesis should have been evident in most oocytes of this mature stage C blue crab (Johnson, 1980), but only one small group of oocytes was vitellogenic, and degenerating oocytes were rather common. The influence of the AG hormones may have been responsible; Payen (1969) reported that AG implantation inhibited vitellogenesis in one of 29 implanted females of another brachyuran, *Rhithropanopeus harrisii.* Neurohormones produced in eyestalk and thoracic ganglia of the crabs *Potomon* and *Eriocheir* influence reproductive condition of females, and increased activity is evident in certain neurosecretory cells of the thoracic ganglion during the reproductive period (Demeusy, 1970). Secretory activity was not morphologically evident in neurosecretory-cell groups on either side of the thoracic ganglion and brain of the blue-crab gynandromorph.

Charniaux-Cotton (1975) summarized the differences between sexual determination in amphipods, isopods, and hermaphroditic natantian decapods on the one hand, and dioecious decapods on the other. She postulated that genes in the first group allow expression of either male or female characters, that the AG is present only in genetic males, and that expression of male characters is brought about by AG hormones. In the second group, experiments to that date indicated that sexual differentiation depended on local inducers, rather than on circulating androgenic hormone produced by the AG, although Charniaux-Cotton considered the AG to be important in regulating changes that occur at puberty. The above concept must be modified because, as mentioned earlier, AG implants cause external masculinization of *P. varians* (Charniaux-Cotton and Cazes, 1979) and complete masculinization of *M. rosenbergii* (Nagamine *et al.*, 1980b); while ablation of the AG causes complete feminization of very young *M. rosenbergii* males (Nagamine *et al.*, 1980a). There is an inverse relationship between the degree of masculinization and the age of the animal at the time of AG implantation in females of *M. rosenbergii* and the various amphipods and isopods studied (Nagamine *et al.*, 1980b). Nagamine and co-workers concluded that earlier attempts to masculinize female decapod crustaceans failed, in part, because there was an insufficient number of molts between AG implantation and termination of the experiments. They also concluded that recently differentiated gonadal systems of both sexes of *M. rosenbergii* and probably other malacostracans are labile, but with age become progressively determined toward the genetic sex of the individual. Therefore, AG implantation in older females would lead to no or only partial masculinization, particularly if the animals molted infrequently.

The existence of bilateral gynandromorphs in species of macrurans and brachyurans shows that sexual differentiation in species capable of gynandromorphism must be either governed differently than in *M. rosenbergii,* or the presence in bilateral gynandromorphs of all the hormonal systems governing expression of sex gives a different final result than artificial presence of the AG alone in genetic females, or artificial absence of AG alone in genetic males. Farmer (1972) emphasized that if the AG of lobsters acted like that of amphipods, isopods, and protandric natantians in controlling maleness, a single AG in a bilateral gynandromorph should not only transform the ovary into a functional testis, but also at least reduce the contrast of external female characters on one side with external male characters on the other side. He suggested that a degree of determinism in the early embryo may be more common than previously thought. Because the AG is present as a morphological entity in male blue crabs at the sixth post-larval molt (Payen *et al.*, 1967), hormones from the AG presumably were present then and later in the gynandromorphic blue crab. Nonetheless, external sexually dimorphic characters were retained fully by the female half even through the pubertal molt. Further, in a gynandromorphic *H. americanus* the gonad adjacent to the crossbridge contained oocytes on the male side (Chace and Moore, 1959). This section completely separated the anterior and posterior lobes of the testis, suggesting a slight dominance of the female hormonal system. In the gynandromorphic blue crab, androgenic hormonal systems were internally dominant. Not only was spermatogenesis taking place through much of the gonad on the female side, but an AVD had been differentiated and vitellogenesis was apparently inhibited.

The existence of bilateral gynandromorphs demonstrates that masculinizing action of the AG in *Homarus, Nephrops,* and *Callinectes* must be tempered by other parts of the hormonal systems, and possibly by early determinism. Eventual form of externally expressed sexual characters must be decided very early during morphogenesis in these genera. The morphological development of the animals before their capture is not known, but their final morphological condition shows that mature external female characters are expressed on the female side despite the presumed presence of androgenic hormones during most or all of development. Gonadal differentiation in the gynandromorphs was apparently more easily influenced by hormones, but not necessarily toward the male form. Evidently, the place of the AG in sexual differentiation of dioecious decapods is complex and may differ depending on the particular decapod group.

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LITERATURE CITED

CHACE, F. A., JR., AND G. M. MOORE. 1959. A bicolored gynandromorph of the lobster, *Homarus americanus. Biol. Bull.* **116:** 226–231.

CHARNIAUX-COTTON, H. 1975. Hermaphroditism and gynandromorphism in malacostracan Crustacea. Pp. 91–105 in R. Reinboth, Ed., *Intersexuality in the animal kingdom.* Springer-Verlag, Berlin/Heidelberg.

CHARNIAUX-COTTON, H., AND M. CAZES. 1979. Première masculinisation des femelles d'un Crustacé Décapode gonochorique, *Palaemonetes varians* (Leach), par greffes de glandes androgènes. *C. R. Hebd. Seances Acad. Sci. Ser. D* **288:** 1707–1709.

CRONIN, L. E. 1942. A histological study of the development of the ovary and accessory organs of the blue crab, *Callinectes sapidus* Rathbun. Master's Thesis, University of Maryland, College Park. 37 pp.

DEMEUSY, N. 1970. Chez les Décapodes Brachyoures gonochoriques, quelques aspects de la sexualité. *Bull. Soc. Zool. Fr.* **95:** 595–612.

FARMER, A. S. 1972. A bilateral gynandromorph of *Nephrops norvegicus* (Decapoda: Nephropidae). *Mar. Biol.* **15:** 344–349.

GEORGE, M. J. 1963. Note on an abnormality in the penaeid prawn *Metapenaeus monoceros* Fabricius. *J. Mar. Biol. Assoc. India* **5:** 145–146.

HARTNOLL, R. G. 1960. An hermaphrodite specimen of the spider crab *Hyas coarctatus* Leach. *Crustaceana* **1:** 326–330.

JOHNSON, P. T. 1980. *Histology of the blue crab,* Callinectes sapidus. *A model for the Decapoda.* Praeger, New York. 440 pp.

NAGAMINE, C., A. W. KNIGHT, A. MAGGENTI, AND G. PAXMAN. 1980a. Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae), with first evidence of induced feminization in a nonhermaphroditic decapod. *Gen. Comp. Endocrinol.* **41:** 423–441.

NAGAMINE, C., A. W. KNIGHT, A. MAGGENTI, AND G. PAXMAN. 1980b. Masculinization of female *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae) by androgenic gland implantation. *Gen. Comp. Endocrinol.* **41:** 442–457.

OTTO, S. V. 1979. Rare condition found in a Chesapeake Bay blue crab. *Commercial Fisheries News* (Maryland Department of Natural Resources) **12(5):** 1.

PAYEN, G. 1969. Expériences de greffes de glandes androgènes chez la femelle pubère du Crabe *Rhithropanopeus harrisii* (Gould) (Crustacé Décapode). *C. R. Hebd. Seances Acad. Sci. Ser. D* **268:** 393–396.

PAYEN, G., J. D. COSTLOW, JR., AND H. CHARNIAUX-COTTON. 1967. Recherches sur le rôle de la neurosécrétion dans la différenciation sexuelle du Crabe *Callinectes sapidus* Rathbun. *C. R. Hebd. Seances Acad. Sci. Ser. D* **264:** 2148–2151.

TOUIR, A. 1977a. Données nouvelles concernant l'endocrinologie sexuelle des Crustacés Décapodes *Natantia* hermaphrodites et gonochoriques. I. Maintien des glandes androgènes et rôle de ces glandes dans le contrôle des gamétogénèses et des caractères sexuels externes mâles. *Bull. Soc. Zool. Fr.* **102:** 375–400.

TOUIR, A. 1977b. Données nouvelles concernant l'endocrinologie sexuelle des Crustacés Décapodes *Natantia* hermaphrodites et gonochoriques. Maintien des gonies et évolution des gamétogenèses *in vivo* et *in vitro. C. R. Hebd. Seances Acad. Sci. Ser. D* **284:** 2515–2518.

TOUIR, A. 1977c. Données nouvelles concernant l'endocrinologie sexuelle des Crustacés Décapodes *Natantia* hermaphrodites et gonochoriques. Mise en évidence d'un contrôle neurohormonal du maintien de l'appareil genital mâle et des glandes androgènes exercé par le protocérébron médian. *C. R. Hebd. Seances Acad. Sci. Ser. D* **285:** 539–542.

Reference: *Biol. Bull.* **161**:246–269. (October, 1981)

THE EFFECTS OF PHYSICAL VARIABLES AND ACCLIMATION ON SURVIVAL AND OXYGEN CONSUMPTION IN THE HIGH LITTORAL SALT-MARSH SNAIL, *MELAMPUS BIDENTATUS* SAY.[1]

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Abstract

The physiological ecology of the salt-marsh pulmonate gastropod, *Melampus bidentatus* Say, was investigated in specimens from the Little Sippewisset salt-marsh, Cape Cod, Massachusetts. *M. bidentatus* is tolerant of submergence, surviving 2–3 days at 20°C and 14 days at 10°C in 25%–100% seawater (SW). Snails acclimated at 10°C tolerated temperatures of 32.2°C–37.4°C, and snails acclimated at 20°C tolerated 35.2°C–40.7°C, when submerged in 0%–100% SW. LD $(T)_{50}$ in air was 44.5°C (10°C acclimated) and 44.7°C (20°C acclimated). Aerial weight-specific O_2 consumption ($\dot{V}O_2$) was 3–5 times higher than aquatic rates. Aerial and aquatic $\dot{V}O_2$ was regulated ($Q_{10} < 1.5$) over the ambient temperature range in both acclimation groups. The $\dot{V}O_2$ of 10°C acclimated individuals was lower than 20°C acclimated specimens, a pattern of "reverse" acclimation associated with energy stores conservation. O_2 debt occurred after 5 h anoxia. In addition, *M. bidentatus* compensates $\dot{V}O_2$ for hypoxia by increasing both uptake rates and the degree of O_2 regulation of $\dot{V}O_2$ during decreasing O_2 concentrations. *M. bidentatus* displays a ciradian rhythm associated with desiccation pressures, and thus $\dot{V}O_2$ is maximal during evening and minimal during daylight hours. Under the selection pressures associated with longterm aerial exposure in the high littoral salt-marsh environment, *M. bidentatus* has evolved the vast majority of physiological adaptations required for terrestrial life, except for its aquatic egg-masses and planktonic development.

Introduction

Pulmonate snails of the genus *Melampus* live in the highest levels of the marine intertidal and are quite literally amphibious. *Melampus bidentatus* Say is found, often abundantly, in the higher levels of salt-marshes along the Atlantic coast of North America. In its reproductive activities, *M. bidentatus* is a primitive marine snail, spawning large numbers of small eggs which yield planktonic veliger larvae on hatching. Thus, all aspects of reproduction and early life-history have to be temporally "fitted" to the 2-week periodicity of spring tides (Russell-Hunter *et al.*, 1972). In contrast, in its respiration, this species is a land snail breathing air though

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Abbreviations: HCT, heat coma temperature; L:D, light:dark (ratio); $LD(T)_{50}$, lethal dose (level) of temperature for 50% of experimental sample; MBL, Marine Biological Laboratory; SW, seawater; $\dot{V}O_2$, weight-specific O_2 consumption rate.

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pneumostome and lung, with no vestige of a ctenidium (gill) in its pallial organization. In this part of its physiology, *Melampus* can best be regarded as an air-breathing snail colonizing the high littoral environment and subjected to variable (but usually short) periods of submergence, variable salinities, and generally high and variable environmental temperatures. This provides a somewhat different adaptational background for the respiratory schesis of *Melampus* than that of the various species of littorinids, gill-bearing marine prosobranch snails, which show colonization of successively higher levels in the intertidal (McMahon and Russell-Hunter, 1977). The investigations reported here on the physiology of *Melampus* assess capacity and resistance responses to temperature and salinity stresses (corresponding to those experienced in the high littoral salt-marsh), and measure survivorship and rates of oxygen uptake at a variety of temperatures and salinities. Both respiratory and survivorship studies involved comparisons between stocks acclimated at different temperatures, and both aquatic and aerial respiration rates were measured.

MATERIALS AND METHODS

Field collections

Specimens of *Melampus bidentatus* were collected from the upper levels (*Spartina-Juncus-Distichlis* zone) of Little Sippewisset salt marsh on Buzzards Bay, just north of Woods Hole, Cape Cod, Massachusetts. Details of the physical and biotic environment of this natural *Melampus* population are provided in Apley (1970) and Russell-Hunter *et al.* (1972). Two collections each of more than 200 individuals were made, on 5 June and 9 June 1975, both at 1430 h. Ambient air temperature was approximately 17°C, surface water temperature in marsh pools 25°C, and subsurface (6 cm) water temperature 23°C, during these two collecting periods. All specimens of *Melampus* were taken at low tide from the muddy marsh surface about the bases of the spike-grass, *Distichlis spicata.* Egg masses of *Melampus* were abundant indicating the population was in a semilunar cycle of egg-laying (see Apley, 1970, and Russell-Hunter *et al.*, 1972, for a detailed discussion of life-cycle and reproduction in *Melampus*). Specimens were carried to the laboratory in insulated jars immediately after collection and placed in 20.3-cm-diameter glass culture bowls (approximately 100/bowl) previously lined with two sheets of 18.5 cm diameter filter paper soaked in full strength seawater. The wet lining was replaced every 3 days throughout the experimental period. The specimens of *Melampus* were divided into two groups, maintained in incubators at 10°C (±0.5°C) and at 20°C (±0.5°C) until utilized in experiments. Both groups were fed an excess of dried lettuce. Neither showed any significant mortality after maintenance under these conditions for more than 50 days.

Submergence tolerance

Melampus is an amphibious pulmonate, primarily air-breathing, being submerged only at high water of spring tides. To determine submergence tolerance in this species, one portion of the sample collected on 9 June 1975 was maintained in air for 2 days after collection at room temperature (21–25°C). Subsequently, subsamples of 10 specimens each were placed in 30 ml of MBL (Marine Biological Laboratory) artificial seawater at five salinities: 100% SW (salinity = 31‰), or 75% (=23.25‰), or 50% (=15.5‰), or 25% (=7.75‰) (see Cavenaugh, 1956, for a detailed account of the chemical composition of MBL artificial seawater), or in Falmouth tap-water previously aerated for 3 days (= <0.2‰).

Nylon screens of 0.1 mm mesh size were secured with plastic snap rings in 40 ml centrifuge tubes (outside diameter = 3.85 cm, height = 11.8 cm) just below the water surface (30 ml volume), preventing aerial emergence by the snails but allowing free gas exchange across the air-water interface. Groups of snails were maintained submerged in this manner at either 10° or 20°C (±0.5°C) for 1, 2, 3, 4, 6, 8, 10, or 14 days. After each submergence period, all water was emptied from the tubes and the snails allowed a 24 h aerial recovery period. The number of snails attached to the walls of each tube then were recorded as alive, and those unable to reattach were recorded as dead.

Effects of salinity on thermal tolerance

The thermal tolerance of submerged snails was determined as mean "heat coma temperature" (HCT) after the method of McMahon (1976) used for a similar sized freshwater pulmonate snail, *Physa virgata.* Heat coma is a reversible condition characterized in molluscs by loss of nervous integration. It is manifested in *Melampus* by cessation of locomotory activity, a ventral-lateral curling of the foot, and inability to remain attached to the walls of the glass tube, a response similar to that of *Physa virgata* (McMahon, 1976; McMahon and Payne, 1980). The basic experiment consisted of placing five specimens in each of four to six 40-ml centrifuge tubes (as described for submergence experiments) with 30 ml of the aquatic medium under test (100%, 75%, 50%, or 25% MBL artificial seawater, or tap-water). The tubes were sealed with porous foam plugs that prevented snails from emerging. Temperatures in the tubes were monitored with a YSI Model 43-Td Tele-Thermometer. Water temperatures in the tubes initially equaled that of acclimation (10°C or 20°C). They were then raised in a block heater 1 degree every 5 min (±0.5 min), a rate of increase that keeps the body temperature of marine littorinid snails far more massive than *Melampus* close to that of the medium without time lag (Broekhuysen, 1940; Evans, 1948). At each 1-degree increase in temperature the number of specimens entering "heat coma" was recorded. Death due to thermal stress usually occurred 3–4°C above HCT in *Melampus.*

In a preliminary experiment, snails from the field were maintained in air at room temperature (21–25°C) as described for 2 days. The shell lengths of 25 individuals were measured to the nearest 0.1 mm with a dial caliper. These measured snails were placed in six 40-ml centrifuge tubes (five tubes of four snails and one tube of five) in size groupings that allowed individuals to be identified visually by shell length. The remaining sample (of about 400 snails) was divided evenly, and the groups maintained at 10°C or 20°C. Subsamples of 20 individuals from each group were tested in full strength MBL seawater after 4, 8, 12, and 16 days and mean HCTs determined. After 19–20 days, mean HCT was also determined for subsamples of 20 individuals from each group in 75%, 50%, 25% MBL artificial seawater and tap-water. It proved impossible to determine the HCT of specimens of *Melampus* in air, as all snails withdrew into the shell at higher temperatures. Therefore, the upper lethal limit of snails in air was determined as the $LD(T)_{50}$, or the lethal dose (level) of temperature for 50% of the sample, for groups acclimated to 10°C or 20°C for 24 days. Five individuals from each group were placed in each of eight 40-ml centrifuge tubes closed with porous foam plugs and placed in a block heater. Temperature was then raised from room temperature (22°C) 1 degree every 5 min. After a temperature of 41°C was reached, and at each subsequent 1°C increase in temperature up to 49°C, one tube each of 10° and 20°C acclimated snails was removed. All snails were allowed 24 h to recover. The number of snails reattached to walls of each tube then were recorded as alive, while those not attached were recorded as dead.

Respiratory response to temperature

Oxygen consumption rates were determined with Clark-type electrodes (YSI Model-53 polarographic silver-platinum oxygen electrodes) used in chambers modified with a glass annulus (Burky, 1977), utilizing the methods for small gastropods described in McMahon (1973) and McMahon and Russell-Hunter (1977, 1978). Respiration chambers were maintained at constant temperature (±0.05°C) with a Haake type-Fe constant temperature circulator. A Honeywell Electronik-16 strip chart recorder was used to monitor changes in chamber oxygen concentration. All oxygen consumption rates were determined for subsamples chosen to have shell lengths within ±2.5 mm of a chosen median value (numbers of individuals in each subsample depending on median shell length). In general, subsamples for each set of experiments were representative of the size range of adult snails in the Little Sippewisset population.

Six subsamples of snails acclimated to 10°C or to 20°C for 20–40 days were used to determine aquatic respiratory response to increasing temperature at three salinities (100% and 50% MBL seawater, and tap-water). Each subsample was placed in the chamber with 4 ml of the appropriate medium at the temperature of acclimation, with a small amount of streptomycin added to inhibit bacterial growth. The respiration chamber temperature was then lowered slowly (1°C/5 min) to 5°C. The oxygen probe was temperature equilibrated in a blank chamber, with the appropriate medium plus streptomycin but without snails (none of the blanks showed significant O_2 consumption). After this stabilization in the blank chamber, the probe was placed in the experimental chamber. Oxygen uptake rate of each subsample was then continuously monitored from near full air saturation with oxygen (PO_2 ca. 140–160 torr) over the first 10% decrease in O_2 tension, or for 1 h if less than 10% had been utilized. This procedure was repeated at 5°C intervals from 5–50°C, to above the upper lethal temperature.

Aerial oxygen consumption rates were measured by modifying the YSI oxygen probe with a neoprene O-ring to form an airtight seal against the walls of the chamber, and by running a 1.0 mm diameter plastic vent tube through the plastic plunger of the electrode (after McMahon and Russell-Hunter, 1977). These modifications allowed insertion of the probe into the chambers to form enclosed volumes of 2 ml. Six subsamples from each of the 10° and 20°C acclimation groups were placed in the chamber with a granule of silica-gel drying agent to prevent moisture condensation on the electrode membrane. The temperature then was gradually lowered from each acclimation temperature to 5°C. The electrode was temperature equilibrated with the vent tube open for 25 min. The probe then was placed in the experimental chamber for 10 min, with the vent tube open. The vent was then sealed and the change in oxygen concentration monitored for 1 h, or until 10% of the oxygen in the chamber was consumed. These measurements were repeated in 5°C intervals at increasingly higher temperatures to 50°C. At the end of each set of determinations, the volume of the experimental individuals and of the silica-gel granule were measured by fluid displacement. By subtraction of their volume from the initial 2 ml chamber volume, the experimental air volume was determined.

Respiratory response to hypoxia

The respiratory response of *Melampus* to hypoxia was determined by methods previously utilized for small molluscs (McMahon, 1972, 1973; McMahon and Russell-Hunter, 1978). The oxygen consumption rates of six subsamples of snails from each of the 10° and 20°C acclimation groups were monitored continuously with declining oxygen concentration in full strength MBL seawater (salinity = 31‰) at 20°C from near full air saturation (PO_2 ca. 140–160 torr) to an oxygen tension at which all uptake ceased. The method of measurement was as described previously for determinations of oxygen consumption rates at full air saturation except that monitoring continued through the entire range of oxygen concentrations over which any uptake occurred. Oxygen uptake rates for each subsample were computed for each 10% decrease in oxygen concentration from near full air saturation with oxygen until an oxygen tension of 30% of air saturation was reached. Thereafter uptake rates were determined for every 5% decrease in oxygen tension until consumption ceased at <3% of air saturation with oxygen ($PO_2 < 4.8$ torr).

Posthypoxic respiratory response in *Melampus* was determined by methods described for other small gastropods (McMahon, 1973; McMahon and Russell-Hunter, 1978). The oxygen uptake of two groups of snails acclimated to 20°C for 49 days was monitored at 20°C from near full air saturation at 20°C in full strength MBL seawater to an oxygen tension at which oxygen uptake ceased. The subsamples were then maintained at this oxygen tension (<3% of air saturation, $PO_2 < 4.8$ torr) for 5 h, after which the chamber water was fully reaerated. Thereafter, the two subsamples were treated differently. The first was used to determine the occurrence of any oxygen debt repayment. Oxygen consumption rates were monitored at near full air saturation immediately after reaeration, at 20 min intervals for 2 h, at 1 h intervals for 4 h, and then at 2–3 h intervals for the remaining 22 h after aeration. After this 28 h period at full air saturation, the oxygen uptake rates of this subsample were again determined over the full range of oxygen concentrations from near air saturation to the oxygen tension at which uptake ceased.

The second subsample was also continuously monitored for O_2 uptake from full air saturation with oxygen to the tension at which uptake ceased, but this was done immediately upon reaeration after 5 h of hypoxia. After this return to full air saturation with oxygen, oxygen consumption rates were measured with declining oxygen concentration at 5 h intervals, for a 25 h period after the initial 5 h period of hypoxia.

Diurnal and tidal influences on oxygen consumption

Diurnal and tidal influences on oxygen consumption of *Melampus* were investigated using two groups of snails. The first was collected in the field at Little Sippewisset during a set of peak spring tides on 9 August 1975 and reutrned to the laboratory for immediate experiment. The second group had been collected from little Sippewisset on 9 June 1975 and maintained in the laboratory without tidal influences for 46 days at 20°C (±0.5°C) and on a 16L:8D cycle before testing began along with the freshly collected group.

The experimental procedure was the same for both groups. Three subsamples of individuals with similar shell lengths were selected from each group. Their aerial oxygen uptake rates were determined as described previously, at approximately 1 h intervals over at least a 24 h period beginning at 0900 h. The two groups were so tested within 3 days of each other.

Computation of oxygen uptake rates

For all determinations of oxygen consumption, uptake rates were expressed as mean $\mu l\ O_2/(\text{animal} \cdot h)$ for each subsample. After each determination, the individuals in the subsample were blotted on filter paper and wet weighed to the nearest 0.1 mg. The mineral component of the shell was then removed by dissolution in 10% (by volume) nitric acid (after McMahon, 1973, 1975). The remaining flesh was then carefully separated from the periostracal component of the shell and dried to constant weight in a 100°C drying oven (>24 h at 100°C). The mean per animal oxygen consumption rates of each subsample were then divided by their mean dry flesh weight to give weight specific uptake rates $[(\mu l\ O_2/(mg \cdot h)$ or $\dot{V}O_2]$.

RESULTS

Submergence tolerance

Temperature had a more pronounced effect on tolerance of submergence than did salinity (Table I). Specimens of *Melampus* at 20°C survived only 1 day in tapwater, 2 days in 100%, 50%, and 25% seawater, and 3 days in 75% seawater. Those maintained at 10°C survived 8 days in tap-water and up to 14 days in 100% to 25% seawater (Table I).

Effect of salinity on thermal tolerance

The heat coma temperature (HCT) of *Melampus* did not show significant correlation with size measured as shell length ($r = 0.36$, $P > 0.1$, N = 25). Therefore, mean values of HCT with their SDs, SEs, and 95% confidence intervals were computed. *Melampus* appears to be able to make substantial acclimatory adjustments in HCT to compensate for sustained shifts in ambient temperature. The mean HCT of freshly collected specimens (ambient air temperature = 17°C) in full strength seawater was 38.8°C (SD = ±1.12°C, N = 25) (Fig. 1). After 12 days at 20°C this value rose to 40.8°C (SD = ±1.51, N = 20) after which no significant change ($P > 0.05$) in mean HCT occurred. After 12 days at 10°C mean HCT fell to 36.3°C (SD = ±1.75°C, N = 20) after which no significant variation

TABLE I

Effects of temperature and salinity on the survival of Melampus bidentatus *during prolonged submergence.*

	Per cent surviving									
	10°C Acclimated					20°C Acclimated				
Days submerged	100% SW	75% SW	50% SW	25% SW	Tap-water	100% SW	75% SW	50% SW	25% SW	Tap-water
1	100	100	100	100	100	100	100	100	100	100
2	90	100	100	100	100	100	100	100	100	0
3	100	100	100	100	100	0	100	0	0	0
4	100	100	100	100	90	0	0	0	0	0
6	100	100	100	100	90	0	0	0	0	0
8	100	90	90	100	90	0	0	0	0	0
10	100	100	100	40	0	0	0	0	0	0
14	80	90	100	90	0	0	0	0	0	0

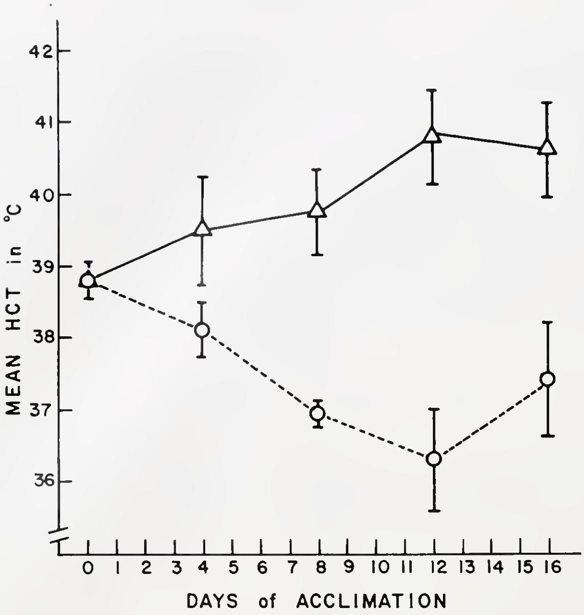

FIGURE 1. Heat coma temperature (HCT) in *Melampus bidentatus* ,and its acclimation with time. The vertical axis shows mean HCT in degrees centigrade. The horizontal axis represents the duration of the temperature acclimation period in days. All snails were freshly collected from the Little Sippiwisset Marsh at day zero (ambient air temperature = 17°C). The open circles connected by dashed lines are mean HCT values of snails held at 10°C, and the open triangles connected by solid lines, mean HCT values of similar snails held at 20°C. Each point is the mean value for 20–30 individuals, and the vertical bars represent 95% confidence limits about mean HCT values.

in HCT ($P > 0.05$) occurred (Fig. 1). Therefore, acclimation periods greater than 20 days were used for all subsequent tests of temperature tolerance and of oxygen consumption. Mean HCT values of 20°C-acclimated snails were significantly higher ($T \geq 2.227$, $P < 0.05$) than those acclimated to 10°C, averaging 4.93°C higher over all salinities tested (SD = ±2.62, range = 3.01–9.45, N = 5). This yields a rate of HCT acclimation per degree increase in acclimation temperature of 0.49°C (Table II).

A one-way analysis of variance revealed that salinity also had a highly significant effect on HCT in *Melampus* at both acclimation temperatures (10°C accli-

TABLE II

Mean heat coma temperature (HCT), standard deviation (SD), standard error (SE), 95% confidence limit (95%) and sample size (N) in relation to salinity and temperature in Melampus bidentatus.

	10°C Acclimated					20°C Acclimated				
Salinity	HCT (°C)	SD	SE	95%	N	HCT (°C)	SD	SE	95%	N
100% SW	37.44	±1.62	±0.38	±0.75	18	40.65	±1.53	±0.35	±0.67	20
75% SW	35.26	±2.47	±0.57	±1.11	19	39.90	±1.25	±0.28	±0.55	20
50% SW	35.43	±1.74	±0.47	±0.91	14	39.75	±1.89	±0.42	±0.83	20
25% SW	31.30	±3.54	±0.79	±1.55	20	40.75	±2.71	±0.61	±1.19	20
Tap-water	32.20	±4.65	±1.04	±2.04	20	35.21	±3.52	±0.81	±1.58	19

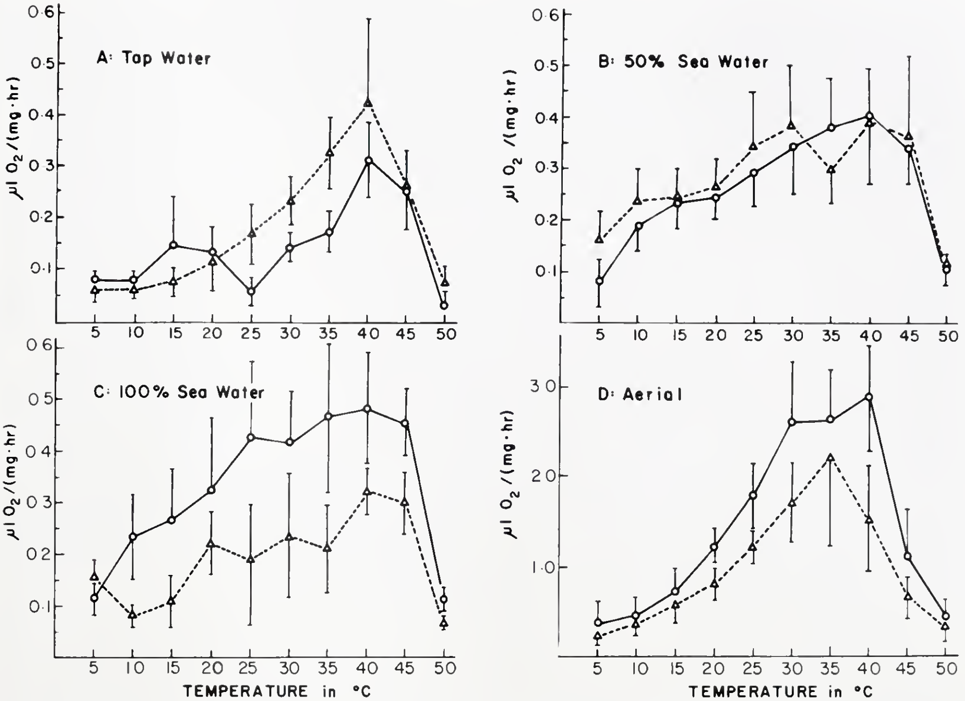

FIGURE 2. Temperature and salinity effects on aerial and aquatic weight-specific oxygen consumption rates ($\dot{V}O_2$) in *Melampus bidentatus*. Open circles connected by solid lines, mean $\dot{V}O_2$ [in $\mu l\ O_2$/(mg dry tissue wt·h) at STP] of 20°C-acclimated individuals and open triangles connected by dashed lines, mean $\dot{V}O_2$ of 10°C-acclimated individuals. A, Mean $\dot{V}O_2$ in tap-water. B, Mean $\dot{V}O_2$ in 50% seawater (salinity = 15.5‰). C, Mean $\dot{V}O_2$ in 100% seawater (salinity = 31.0‰). D, Mean $\dot{V}O_2$ in air (note difference in scale of the vertical axis). The vertical bars represent 95% confidence limits about the mean $\dot{V}O_2$ values for six replicate subsamples (limits not indicated if they overlap the mean $\dot{V}O_2$ value of the other acclimation group at the same temperature).

mated, $F_{4,86} = 12.25$, $P < 0.001$; 20°C acclimated, $F_{4,94} = 18.96$, $P < 0.001$). A Student-Newman-Keuls test revealed that mean HCT in 10°C-acclimated individuals in 100% seawater was significantly greater ($P < 0.05$) than mean HCT in all other aquatic media. Mean HCT values in tap-water and 25% seawater did not differ significantly ($P > 0.05$), nor did values for 50% and 75% seawater. In contrast, HCT values for 50% and 75% seawater were significantly greater ($P < 0.05$) than the values for 25% seawater and tap-water. For 20°C-acclimated individuals mean HCT in tap-water was significantly less ($P < 0.05$) than those in salinities between 25% and 100% seawater, which were not significantly different ($P > 0.05$) from each other.

The $LD(T)_{50}$ of *Melampus* in air was 44.5°C for 10°C-acclimated individuals and 44.7°C for those acclimated to 20°C. All 10°C-acclimated snails survived at 44°C in air but none at 45°C. Those acclimated to 20°C died at either 45°C or 46°C in air with none surviving at 47°C.

Respiratory response to temperature and salinity

Figure 2 shows the change in mean weight-specific oxygen rate in $\mu l\ O_2$/(mg·h) ($=\dot{V}O_2$) of 10°- and 20°C-acclimated snails under differing conditions of temperature and salinity. Least squares linear regression analysis of logarithmic transformations of values in $\mu l\ O_2/(\text{animal} \cdot h)$ versus mean dry weight values revealed that only 9 out of 80 such determinations showed any significant ($P < 0.05$) correlation between dry tissue weight and oxygen uptake rate. (In part, this was due to the limited size range of adult snails used from in the Little Sippewisset population.) Therefore, all oxygen consumption rates were computed as means combining all repeated individual determinations.

A two-way analysis of variance of the effect of test temperature and acclimation temperature on mean $\dot{V}O_2$ revealed that test temperature had a significant effect ($F_{9,27} > 4.8$, $P < 0.01$) in all four media tested. Acclimation temperature only affected oxygen uptake rates significantly in 100% seawater ($F_{1,9} = 12.8$, $P < 0.001$) and in air ($F_{1,9} = 10.5$, $P < 0.01$). Further analysis by t test of the mean $\dot{V}O_2$ values of the 10°- and 20°C-acclimated snails at each salinity-temperature combination tested revealed that significant differences ($P < 0.05$) occurred only at 25°, 30° and 35°C in tap-water, only at 5°C in 50% seawater, at 5°, 10°, 15°, 25°, 35°, 40°, 45°, and 50°C in 100% seawater and at 20°, 25°, 30°, and 40°C in air. Figures 2c and d show that the oxygen uptake rates of 20°C-acclimated individuals (both in 100% seawater and air) were always higher than those of 10°C-acclimated individuals, except at 5°C in 100% SW. Such elevation of rates in individuals acclimated to higher temperatures has been referred to as "reverse" or "paradoxical" acclimation (Berg, 1951, 1952, 1953; Proser, 1973).

A two-way analysis of variance of the effect of medium (aerial vs. aquatic) and test temperature on mean $\dot{V}O_2$ revealed that the medium significantly affected oxygen consumption rate ($F_{3,27} > 15.0$, $P < 0.001$). In both 10°- and 20°C-acclimated snails, $\dot{V}O_2$ was not significantly different ($P > 0.05$) among all three aquatic media, but was significantly greater in air ($P < 0.05$) than in the aquatic media (as analyzed by Tukey's test for multiple pair-wise comparisons).

Table III shows values of Q_{10} corresponding to changes in $\dot{V}O_2$ over 5°C intervals for each of the four test media (100% and 50% seawater, tap-water and air). A two-way analysis of variance comparing the effects of temperature (over the series of 5°C intervals) and medium showed that while temperature significantly affected Q_{10} ($F_{8,24} > 2.58$, $P < 0.05$) in both 10° and 20°C acclimation groups, the

Table III

Mean Q_{10} values for the change in the mean weight specific oxygen consumption in individuals of Melampus bidentatus *(acclimated to 10°C or 20°C) over 5°C intervals from 5°C to 50°C in 100% and 50% sea water, tap water, and in air.*

Temperature range in °C	10°C Acclimated				20°C Acclimated			
	100% SW	50% SW	Tap-water	Air	100% SW	50% SW	Tap-water	Air
5–10	0.253	2.086	1.000	2.527	4.291	5.724	0.976	1.426
10–15	1.834	1.070	1.552	2.504	1.295	1.936	3.294	2.640
15–20	4.227	1.199	2.250	1.944	1.479	0.854	0.819	2.943
20–25	0.734	1.606	2.224	2.293	1.739	1.444	0.647	2.136
25–30	1.533	1.254	1.895	1.945	0.954	1.372	1.862	2.143
30–35	0.805	0.601	1.989	1.716	1.249	1.235	1.453	1.018
35–40	2.322	1.733	1.651	0.464	1.070	1.125	3.203	1.190
40–45	0.873	0.843	0.359	0.186	0.887	0.703	0.640	0.148
45–50	0.093	0.092	0.085	0.234	0.056	0.102	0.013	0.152

type of medium did not ($F_{3,24} < 0.98$, $P > 0.1$). Similarly, a two-way analysis of variance of the effects of test temperature and acclimation temperature on Q_{10} revealed that acclimation temperature did not significantly affect the respiratory response to increasing temperature in any of the four media tested ($F < 1.08$, $P > 0.1$). This result is supported by the lack of any significant difference ($P < 0.05$) between mean Q_{10} values of 10°- and 20°C-acclimated individuals for any of the pairs of 36 medium-test temperature combinations (as displayed in Table III) analyzed.

The mean sequential Q_{10} values for 5°C intervals throughout the normal 10°–40°C ambient temperature range of *Melampus* were: 1.909 (SD = ±1.288) in 100% seawater; 1.244 (SD = ±0.404) in 50% seawater; 1.937 (SD = ±0.288) in tap-water; and 1.813 (SD = ±0.718) in air for 10°C-acclimated individuals. Corresponding values for 20°C-acclimated snails were: 1.301 (SD = ±0.285) in 100% seawater; 1.328 (SD = ±0.363) in 50% seawater; 1.725 (SD = ±1.210) in tap-water; and 2.016 (SD = ±0.769) in air. In air, the $\dot{V}O_2$ of 10°C-acclimated snails generally increased with temperature to 35°C. Above this, the rate declined due to temperature stress. In 20°C-acclimated individuals, $\dot{V}O_2$ increased to 25°C, remained stable through 40°C (Q_{10} between 25° and 40°C = 1.374), and then declined (Fig. 2d). Similar regulation of $\dot{V}O_2$ with increasing temperature between 25° and 45°C (Q_{10} = 1.03) is very apparent in 20°C-acclimated snails in 100% seawater (Fig. 2c).

Respiratory response to hypoxia

Figure 3 shows the mean $\dot{V}O_2$ values (n = 6) of 10°- and 20°C-acclimated individuals with decreasing O_2 concentration in 100% seawater at 20°C. $\dot{V}O_2$ values with decreasing oxygen concentration for each of six individual subsamples from the 10°C or 20°C acclimation groups were expressed as fractions of the rate at

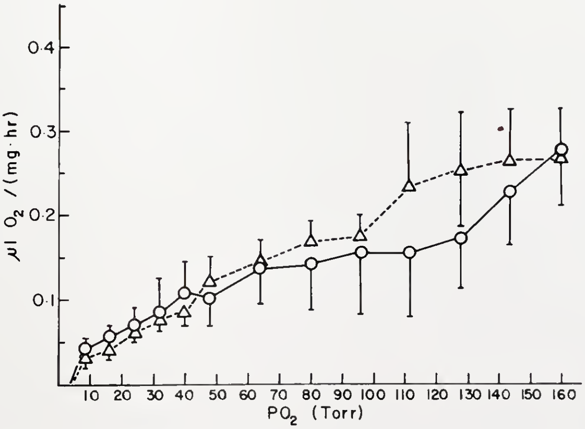

FIGURE 3. Effect of declining oxygen concentration on mean weight-specific oxygen consumption rate ($\dot{V}O_2$) of *Melampus bidentatus* in seawater (salinity = 31.0‰), from full air saturation with oxygen (PO_2 = 159 torr). The horizontal axis represents the partial pressure of oxygen in torr and the vertical axis, mean $\dot{V}O_2$ [in μl O_2/(mg dry tissue wt·h) at STP]. The open circles connected by solid lines are $\dot{V}O_2$ values of 20°C-acclimated individuals and the open triangles connected by dashed lines, $\dot{V}O_2$ values of 10°C-acclimated individuals. The vertical bars represent 95% confidence limits about the mean $\dot{V}O_2$ values for six replicate subsamples (limits not indicated if they overlap the mean $\dot{V}O_2$ value of the other acclimation group at the same temperature).

air saturation with oxygen and fitted versus PO_2 in torr to a quadratic equation. The resultant quadratic coefficient (b_2) is a predictor of the degree of regulation (independence) of oxygen uptake with declining oxygen concentration. It becomes an increasingly large negative value as the degree of regulation increases (Mangum and Van Winkle, 1973). Expressed as $b_2 \times 10^3$, the means of these values for 10°C-acclimated ($b_2 \times 10^3 = -0.0183$, SD = ± 0.0098, N = 6) and 20°C-acclimated individuals ($b_2 \times 10^3 = -0.0067$, SD = ± 0.0337, N = 6) were not significantly different ($T = 0.817$, $P > 0.1$). Adjusted $\dot{V}O_2$ values of all six subsamples were fitted to a single quadratic equation, to yield $b_2 \times 10^3$ values of -0.0175 and -0.004 for the 10°- and 20°C-acclimated groups, respectively. When based on the mean values presented in Fig. 3, $b_2 \times 10^3$ values were -0.0174 (10°C acclimated) and -0.0021 (20°C acclimated). As such, all three methods of computation of average $b_2 \times 10^3$ values yielded similar results, with values ranging between -0.0021 and -0.0183. This range of values indicated that *Melampus* was unable to regulate oxygen consumption with decreasing oxygen concentration whether acclimated to 10° or 20°C (Fig. 3).

After exposure to hypoxic conditions, *Melampus* appeared to pay an oxygen debt. Figure 4a reveals that $\dot{V}O_2$ at near full air saturation with oxygen ($PO_2 >$ 150 torr) increased to 2.4 times the prehypoxic rate, and remained noticeably elevated for about 2 h after return to full air saturation following 5 h of low oxygen stress. The amount of debt paid, as a volume of oxygen respired, was estimated after the method of McMahon and Russell-Hunter (1978). The portion of each posthypoxic $\dot{V}O_2$ value that represented an increase over the prehypoxic rate of 0.213 $\mu l\ O_2/(mg \cdot h)$ was multiplied by each time span (as fractions of an h) extending from halfway between each measurement and the preceding one to halfway between the measurement and the next, until $\dot{V}O_2$ again fell close to the prehypoxic value. Total oxygen debt was estimated as the volume of oxygen that would have been respired at the prehypoxic rate over the 5 h period of hypoxia. Such computations showed that total debt repayment in *Melampus* was only about 29.5% of the estimated accumulated oxygen debt. After returning to prehypoxic rates at 2 h posthypoxia, $\dot{V}O_2$ again slowly increased reaching 0.616 $\mu l\ O_2/(mg \cdot h)$ by 28 h after the low oxygen stress period (Fig. 4a). Figure 4b shows the $\dot{V}O_2$s with decreasing oxygen concentration of a subsample of *Melampus* before and after 5 h of hypoxia. Before hypoxic stress $\dot{V}O_2$ was not regulated; it decreased proportionately with oxygen concentration (Fig. 3, 4b). A similar response occurred just after return to air saturation from the 5 h of hypoxia, except that uptake rates were more than twice prehypoxic levels, due to payment of the oxygen debt just described. This lack of regulation is reflected by high $b_2 \times 10^3$ values of -0.0176 before and -0.0030 just after the low oxygen stress period. However, from 5–20 h after hypoxia, oxygen consumption not only remained 30%–50% greater than prestress levels at PO_2s greater than 100 torr, but shifted markedly toward increased regulation. The rate of decline in $\dot{V}O_2$ was greatly reduced from full air saturation down to a PO_2 of 35 torr, the critical oxygen tension below which $\dot{V}O_2$ declined acutely with further decrease in PO_2 (Fig. 4b). This increase in the posthypoxic regulatory ability of *Melampus* is manifested by a sizable decrease in $b_2 \times 10^3$ values (5 h posthypoxia, $b_2 \times 10^3 = -0.0500$, 10 h = -0.0014, 15 h = -0.0245, and 20 h = -0.0576) averaging -0.0332. This indicates a shift to moderate or good regulation of oxygen uptake with declining oxygen tension (Mangum and Van Winkle, 1973).

This combination, a general increase in $\dot{V}O_2$ over the full range of PO_2 from air saturation and a shift towards increased regulation of oxygen consumption,

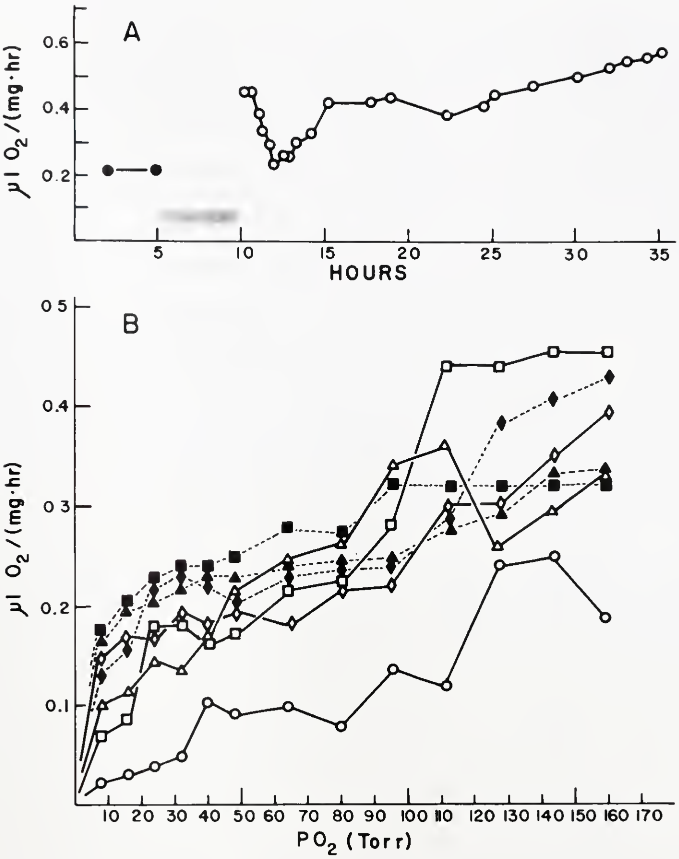

FIGURE 4. The effect of a 5 h period of anoxia on the weight-specific oxygen consumption rate [$\dot{V}O_2$ in µl O_2/(mg dry tissue wt·h) at STP] of *Melampus bidentatus* in 100% seawater at 20°C. A, The effect of anoxia on $\dot{V}O_2$ in fully air-saturated seawater. The horizontal axis is time in hours and the vertical axis $\dot{V}O_2$ as above. The solid circles represent $\dot{V}O_2$ before 5 h of anoxia; and the open circles, $\dot{V}O_2$ for up to 25 h after return to fully air-saturated seawater (PO_2 = 159 torr) from the 5 h of anoxia. B, Effect of five hours of anoxia on $\dot{V}O_2$ with decreasing oxygen concentration from full air saturation (PO_2 = 159 torr). Horizontal axis, partial pressure of oxygen in torr; vertical axis, $\dot{V}O_2$ as above. Open circles connected by solid lines represent $\dot{V}O_2$ with declining oxygen concentrations before anoxia. Open triangles, squares, and diamonds connected by solid lines represent $\dot{V}O_2$ immediately after, 5 h after and 10 h after return to fully air-saturated seawater from anoxia, respectively. Solid triangles, squares and diamonds connected by dashed lines represent $\dot{V}O_2$ 15 h, 20 h, and 25 h after anoxia, respectively.

allows *Melampus* to maintain oxygen uptake rates at or near prehypoxic rates at full air saturation, down to PO_2s as low as 30 torr for 5 to 20 h after 5 h of hypoxia (Fig. 4b). In untreated snails at 30 torr, there is no regulation, and uptake rates run at about 20–40% of poststress rates. By 25 h posthypoxia, the ability of *Melampus* to regulate oxygen consumption appears to be lost ($b_2 \times 10^3 = -0.0012$), but $\dot{V}O_2$ remains elevated over the full range of oxygen tensions tested, allowing continued maintenance of uptake rates close to prestress full air saturation levels at PO_2s as low as 65 torr. In the first subsample, $b_2 \times 10^3$ values remained low and uptake rates elevated after 28 h return from hypoxia at −0.0426. This indicated slightly longer retention of oxygen regulation in this group.

Diurnal and tidal influence on oxygen consumption

The repeated determinations of aerial $\dot{V}O_2$ at 20°C of three subsamples of snails freshly collected from the field, and of those maintained at 20°C in the laboratory on a L:D cycle of 16:8, without tidal influence, were averaged on an hourly basis, and then computed as a running smoothed mean of the average hourly value and their two adjacent values. These smoothed values were then expressed as percentages of the overall mean $\dot{V}O_2$ of each group and plotted against the average time of measurement for each original hourly triplet of determinations (Fig. 5). In freshly collected snails, aerial $\dot{V}O_2$ increased steadily from a low value of 88% of the mean at 1200 h (Eastern Standard Time) to a peak $\dot{V}O_2$ of 117% of the mean at 2359 h. This was followed by decline to 83% to 84% of the mean at 1200 h the following day, after which uptake again began to rise (Fig. 5). The diurnal pattern of $\dot{V}O_2$ variation was apparently reversed in the laboratory-maintained specimens. Peak rates of 125%–129% of mean $\dot{V}O_2$ occurred at 1000–1600 h and then declined to 72%–74% of the mean at 2300–0300 h (the middle of the artificial 8-h dark period). $\dot{V}O_2$ thereafter rose steadily, returning to near the mean value by 1200 h the following day (Fig. 5).

DISCUSSION

These results clearly indicate that, in such aspects of its somatic physiology as respiration and water metabolism, *Melampus bidentatus* is highly adapted to life

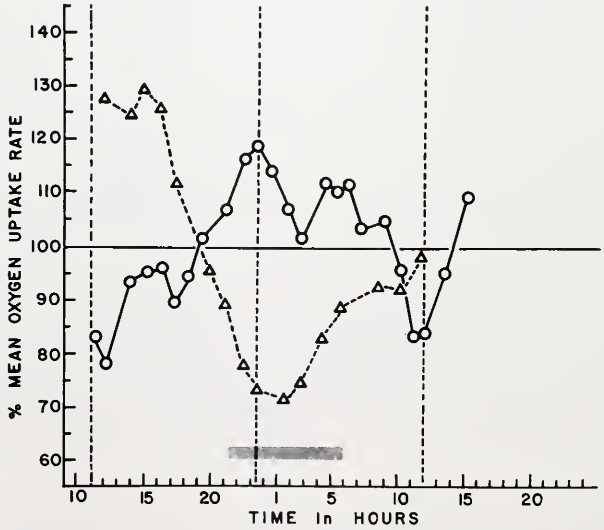

FIGURE 5. Diurnal variation in weight-specific oxygen consumption rate ($\dot{V}O_2$) of *Melampus bidentatus*. Horizontal axis, time in hours from 1000 h, when $\dot{V}O_2$ measurement began, until 1600 h the following day, when the experiment ended. The vertical axis, the per cent value by which smoothed mean values of $\dot{V}O_2$ differ from the mean value of $\dot{V}O_2$ over the entire time course of the experiment (represented by the solid line extending horizontally from the 100% point on the vertical axis). Open circles connected by solid lines, smoothed values of $\dot{V}O_2$ computed from three subsamples of similar sized individuals freshly collected from the Little Sippiwisset Marsh. Open triangles connected by dashed lines, similar smoothed values of $\dot{V}O_2$ for individuals maintained at 20°C and at 16L:8D for 46 days in the laboratory. The shaded horizontal bar indicates the duration of the dark period to which laboratory-maintained individuals were exposed and the dashed vertical lines, the times of peak high tides in the Little Sippiwisset Marsh during the experiment.

in the upper levels of the littoral salt-marsh environment. A primitive pulmonate of the family Ellobiidae, it has lost the ctenidium associated with aquatic respiration and modified the mantle-cavity into a highly vascularized diffusion lung for aerial gas exchange (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Russell-Hunter, 1979; Price, 1976, 1980). This represents adaptation to the high degree of aerial exposure in the upper reaches of the salt-marsh, since it is exposed to air over 95% of the lunar tidal cycle, and is submerged only at peak spring high tides (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; McMahon and Russell-Hunter, 1977; Price, 1976, 1980). Without a gill, the snail's respiratory gas exchange when submerged is cutaneous over its general body surface. It does not appear to use a gas bubble in the mantle-cavity as a physical gill (Russell-Hunter *et al.*, 1972). Submerged oxygen uptake is less than corresponding aerial oxygen uptake at temperatures between 5°C and 45°C (Fig. 2).

It has been proposed that *M. bidentatus*, as a lung-breather, would asphyxiate during even relatively short periods of submergence (Russell-Hunter *et al.*, 1972). The present results indicate that *M. bidentatus* is more tolerant of submergence than previously suspected, surviving (at 20°C) at least 2 days in 100% to 25% SW (3 days in 75% SW) and at least 1 day in tap-water (Table I). Price (1979) reported even greater tolerances, of more than 10 days submerged in 100% SW and up to 3 days in tap-water at 20°C. These periods are far longer than the 1.5–3 h of submergence *M. bidentatus* is reported to experience at high spring tides (Apley, 1967; Russell-Hunter *et al.*, 1972; McMahon and Russell-Hunter, 1977), and may represent a sort of "safety factor" evolved to allow survival under even the most extreme flooding by storm tides or prolonged rainfall. *Amphibola crenata*, a related pulmonate endemic to estuarine mud-flats in New Zealand, appears to be much better adapted to aquatic conditions than *M. bidentatus*. It survives 14 days in freshwater and up to 30 days in seawater, but only 1 day without water (Farnie, 1919), while *M. bidentatus* can survive for at least 3 days in all relative humidities above 50% (Price, 1980).

At 10°C, tolerance of submergence (in 25%–100% SW) is remarkably increased in *M. bidentatus;* 80% to 100% survived on termination of the experiment after 14 days of submergence in seawater and near 100% survived after 8 days in tap-water (Table I). At such lower temperatures (and in salinities above 50% SW), oxygen uptake is reduced by a combination of low temperature effects and respiratory "reverse" acclimation. The O_2 uptake rates of submerged individuals at 10°C are only 25% to 75% of individuals at 20°C (Figs. 2a, b, c). Reduction in oxygen demand could allow individuals submerged at 10°C to survive indefinitely by cutaneous gas exchange in all salinities with no severe osmotic stress.

Melampus bidentatus overwinters by burrowing into the marsh floor or by entering the abandoned burrows of the fiddler crab, *Uca*, (Hausman, 1932; Apley, 1967, 1970; Price, 1976, 1980) to soil depths that remain unfrozen. When covered by spring tides, *Uca* burrows and the peaty marsh soils remain flooded far longer than the marsh surface. During winter months *M. bidentatus* is inactive (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1980), and thus can be exposed to rather long periods of submergence in waters of relatively low ambient temperature. Reduced metabolic rates in cold-acclimated individuals allow survival of much longer periods of submergence with entirely cutaneous gas exchange. During spring and summer *M. bidentatus* returns to the marsh surface and can actively avoid submergence during spring tides by crawling up the stems of marsh grass (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1980).

M. bidentatus spends a portion of its active hours on the high salt-marsh surface and is often directly exposed to sunlight. Such insolation can cause salt-marsh surface temperatures to be several degrees higher than ambient air temperatures. *M. bidentatus* can tolerate high temperatures, as can certain other gastropods of the higher intertidal (Gowanloch and Hayes, 1926; Broekhuysen, 1940; Evans, 1948; Fraenkel, 1961, 1966, 1968; Lewis, 1963; Sandison, 1967; McMahon and Russell-Hunter, 1977). The temperatures at which activity ceased (HCT) in 20°C-acclimated specimens of *M. bidentatus* ranged between 40.7°C in 100% SW and 35.2°C in tap-water. For 10°C-acclimated individuals, the corresponding range was lower (Table II). This decrease in tolerance would suggest that thermal tolerance of submerged individuals is subject to temperature acclimation. In contrast, in air, temperature acclimation did not affect lethal temperatures for *M. bidentatus*. The $LD(T)_{50}$'s of 10°C- and 20°C-acclimated individuals were very similar: 44.5°C and 44.7°C, respectively.

It appears that 10°C-acclimated individuals have somewhat depressed upper lethal temperature limits when submerged. In contrast, 20°C-acclimated snails appear to have nearly equal upper lethal limits in air and water. The reasons for this difference are not entirely clear. Certainly the large amounts of body water which *M. bidentatus* is capable of losing under desiccation stress (Russell-Hunter and Meadows, 1965; Price, 1980) indicate some short-term evaporative cooling in air is possible as a passive response. But the more likely explanation lies in the more complex interactions involving metabolic rates and acclimation, reviewed for marine and estuarine animals by Kinné (1964, 1971), Newell (1976), and Lockwood (1976). There was no significant difference in the HCT of 20°C-acclimated specimens of *M. bidentatus* in 100% to 25% SW (Table II). Regulation of thermal tolerance over a range of salinities is highly adaptive for a high salt-marsh animal which is exposed to extreme variations of both temperature and salinity.

However, when *M. bidentatus* was winter- or cold-conditioned (10°C-acclimated), it lost its ability to modify thermal tolerance levels; HCT decreased proportionately with salinity (Table II). This loss of ability to modify thermal tolerance with changing salinity would not lead to stress over the range of ambient temperatures encountered (in burrows) during the overwintering period.

Overwintering individuals of *M. bidentatus* are also tolerant of freezing temperatures. They can be maintained at −12°C for 3 days with mortality rates ranging from 85.7% for individuals with shell lengths of less than 5 mm to only 26.7% for those with shell lengths greater than 7 mm (Price, 1980; see also Hilbish, 1981). Similar tolerance to freezing has been described in other intertidal molluscs.

Aquatic oxygen consumption of individuals of *M. bidentatus* acclimated at 10°C and 20°C is much less than corresponding aerial rates over all tested salinities and temperatures (Fig. 2), except for 20°C-acclimated individuals in tap-water (an "unnatural" condition poorly tolerated by *M. bidentatus*, see Table I).

It is obvious that a pulmonate like *M. bidentatus*, which has lost the ctenidium and evolved a highly vascularized mantle-cavity as an aerial diffusion lung, and which spends the vast majority of each lunar tidal cycle exposed to air, should show elevated rates of aerial $\dot{V}O_2$ (Fig. 2a–d). In contrast, the aquatic $\dot{V}O_2$ values of prosobranch littorinid snails which retain the ctenidium are generally reported to be greater than (or, at least, similar to) aerial rates of O_2 uptake. Such respiratory patterns have now been described for *Littorina neritoides*, *L. saxatilis* (more properly *L. rudis*), *L. littorea*, and *L. obtusata* (Toulmond, 1967a, b; Raffy, 1933; Fischer *et al.*, 1933; McMahon and Russell-Hunter, 1977). In more aquatic subtidal prosobranchs, aerial $\dot{V}O_2$ is generally much less than aquatic rates of O_2 uptake (McMahon and Russell-Hunter, 1977; McMahon, unpublished data). Because O_2

uptake and activity are predominately aerial in *M. bidentatus*, this species appears far better adapted to terrestrial life than marine high-littoral prosobranchs. A pattern of nearly equivalent aerial and aquatic $\dot{V}O_2$ is also retained by primitive high littoral estuarine pulmonate, *Amphibola crenata*, which is somewhat more aquatic than *M. bidentatus* (see discussion of submergence tolerance above). *Amphibola* is apparently able to maintain equivalent $\dot{V}O_2$ in air or in seawater (Shumway, 1981) but it may not have as great a capacity to tolerate extended periods of aerial exposure as does *M. bidentatus* (Farnie, 1919).

Salinity does not greatly effect $\dot{V}O_2$ in *M. bidentatus* over a range of 0% to 100% SW. *A. crenata* is similar in this respect (Shumway, 1981). Both Kinné (1971) and Schlieper (1971), in their extensive reviews of the effects of salinity on estuarine organisms, state that there are relatively few highly euryhaline species in which metabolism remains independent of salinity. Among prosobranch gastropods only two essentially freshwater species display any salinity independence in O_2 consumption: *Theodoxus fluviatilis* (Lumbye, 1958) and *Potamopyrgus jenkinsi* (Lumbye and Lumbye, 1965; Duncan, 1966; Duncan and Klekowski, 1967). For both *M. bidentatus* and *A. crenata*, such salinity independence of $\dot{V}O_2$ is highly adaptive for life in the highest reaches of salt-marshes or estuaries, where salinities vary acutely with both tidal cycle and rainfall (Lockwood, 1976; Kinné, 1971; Newell, 1976; Schlieper, 1971).

The aerial $\dot{V}O_2$ of 10°C-acclimated individuals of *M. bidentatus* displays a normal response (Prosser, 1973) to temperature, with Q_{10} values falling within the range of 1.5–2.5 (Table III, Fig. 2d). In contrast, aerial $\dot{V}O_2$ in 20°C-acclimated individuals appears to be temperature regulated, with little or no apparent increase in $\dot{V}O_2$ and with Q_{10} values near 1.0 between 30°C and 40°C (Table III, Fig. 2d). Similar regulation in aquatic O_2 consumption occurs at all tested temperature ranges in 50% and 100% SW (Table III, Fig. 2b, c). There is no apparent temperature regulation of aquatic O_2 consumption in either 10°C- or 20°C-acclimated individuals in tap-water (Table III, Fig. 2a). Such temperature regulation of $\dot{V}O_2$ within the normal ambient temperature and salinity range allows maintenance of relatively efficient metabolic rates in an intertidal environment characterized by acute, short-term temperature variations. Similar temperature regulation of $\dot{V}O_2$ has been reported for the intertidal prosobranchs, *Littorina littorea* (Newell, 1973; Newell and Pye, 1970a, b, 1971a, b; Newell and Roy, 1973; McMahon and Russell-Hunter, 1977), and *L. irrorata* (Shirley *et al.*, 1978), the intertidal bivalve, *Mytilus edulis* (Newell and Pye, 1970a, b), and in several other intertidal invertebrates (as reviewed by Newell, 1976, 1979).

In its respiratory schesis, *M. bidentatus* also displays the physiological phenomenon of "reverse" or "inverse" acclimation, whereby 20°C-acclimated snails have higher, *rather than the more normal lower*, values of $\dot{V}O_2$ than do snails acclimated to 10°C, at all test temperatures (for discussions of reverse acclimation see Burky, 1971; McMahon, 1972, 1973; Calow, 1975; Parry, 1978). such reverse respiratory acclimation to temperature has previously been reported in the intertidal limpet, *Patella vulgata* (Davies, 1965), a terrestrial stylommatophoran pulmonate, *Helix pomatia* (Blazka, 1954), four species of basommatophoran ancylid limpets, *Ancylus fluviatilis, Acroloxus lacustris, Ferrissia rivularis,* and *Laevapex fuscus* (Berg, 1951, 1952, 1953; Berg *et al.*, 1958; Calow, 1975; Burky, 1969, 1970, 1971; McMahon, 1972, 1973, 1975). Other than molluscs, reverse acclimation has been reported in barnacles (Barnes *et al.*, 1963), a freshwater gammarid (Krog, 1954), a goldfish (Roberts, 1960, 1966), and a hylid frog, *Pseudocris triseriata* (Packard, 1972).

Many hypotheses have been made regarding the adaptive value of reverse acclimation. These include:

1. Reduction of $\dot{V}O_2$ in overwintering animals is an adaptation to hypoxic conditions during ice cover (Roberts, 1960, 1966; Krog, 1954);
2. The resultant decrease in metabolic rate conserves scarce energy stores in inactive (non-feeding) overwintering individuals (Burky, 1969, 1971; Packard, 1972);
3. The phenomenon is only one manifestation of a more general change in respiratory response involving a shift from oxygen dependence of $\dot{V}O_2$ in warm-acclimated individuals to strict regulation of $\dot{V}O_2$ in cold-acclimated individuals, allowing overwintering animals to maintain higher O_2 consumption rates than summer individuals in their normally hypoxic overwintering microhabitats (McMahon, 1973, 1975);
4. Metabolic rate is directly proportional to growth rates, and, therefore, slow growing overwintering individuals have a lower $\dot{V}O_2$ than fast growing summer individuals (Parry, 1978); and
5. Reverse acclimation and the large seasonal shifts in respiratory rate associated with it are a specific adaptation to the rapidly fluctuating temperatures associated with certain low temperature environments, such as streams and wave-swept littoral regions of lakes (Calow, 1975).

In *Melampus bidentatus*, overwintering individuals are inactive, do not feed, show little growth, and probably survive on accumulated energy stores (Apley, 1967, 1970; Russel-Hunter *et al.*, 1972). However, no direct data suggest tissue "degrowth" in *M. bidentatus*, as occurs in other pulmonates (Russell-Hunter and Eversole, 1976). In the marsh substratum, *M. bidentatus* encounters not only periodic submergence and low temperatures, but also periods of hypoxia resulting from decomposition of buried organic matter (Lockwood, 1976). Under these conditions, reverse acclimation would conserve overwintering energy stores (primarily glycogen in gastropods [Goddard and Martin, 1966]) at the same time reducing the demand for oxygen. Such a reduction is highly adaptive in these hypoxic overwintering conditions, and could be stabilized at levels that could be entirely supported by cutaneous respiration in such torpid overwintering individuals.

As case studies of poikilothermic organisms accumulate, it has become increasingly obvious that inverse or reverse acclimation evolved independently in a variety of species and phyletic groups in response to a series of quite different selective pressures. It cannot be described in terms of a single unified adaptive advantage for all the species in which it occurs, although its evolution may always have involved a reversal of pre-existing signals for genetically patterned control mechanisms. In *M. bidentatus*, reverse acclimation almost certainly involves a sort of "torpor" that conserves energy stores, as earlier hypothesized for freshwater limpets (Burky, 1969, 1971) and for hylid frogs (Packard, 1972). It may also function to lower oxygen demands at times when oxygen availability is reduced due to hypoxic conditions, and a switch to cutaneous respiration in overwintering individuals as has been hypothesized for goldfish (Roberts, 1960, 1966) and gammarids (Krog, 1954).

As *M. bidentatus*, whether cold or warm acclimated, has little or no ability to regulate $\dot{V}O_2$ in decling O_2 concentrations (Fig. 3), reverse acclimation in this species is *not* explainable as a shift to greater regulation in winter-conditioned individuals, as an adaptation of hypoxic overwintering conditions, as McMahon (1973) claimed for a freshwater pulmonate limpet, *Laevapex fuscus*. The overwintering environment of *M. bidentatus* cannot be characterized as one of highly fluctuating temperatures, and thus cannot correspond to Calow's (1975) hypothesis on the adaptive advantage of reverse acclimation in freshwater gastropods. The phenomenon does not appear to be associated with any seasonal changes in growth rate and food availability, as hypothesized by Parry (1978). Indeed, an excess of food was provided to both 10°C and 20°C acclimation groups throughout our experiments. Other molluscs do display seasonal patterns of acclimation, with slow growing cold-conditioned overwintering individuals having higher values of $\dot{V}O_2$ at any one temperature than do the faster growing warm-conditioned spring individuals. This clearly occurs in the freshwater clam *Pisidium walkeri* (Burky and Burky, 1976; Burky, personal communication).

M. bidentatus is apparently not as well adapted to temporarily hypoxic conditions as are several species of freshwater pulmonates and marine prosobranchs that display little or no oxygen debt after long periods of anoxia (von Brand, 1946; von Brand and Mehlman, 1953; von Brand *et al.*, 1950; McMahon, 1973; McMahon and Russell-Hunter, 1974, 1978; deZwaan and Wijsman, 1976). Unlike these other species, *M. bidentatus* does not regulate $\dot{V}O_2$ with declining O_2 concentration (see Fig. 3). It has to repay a considerable O_2 debt, maintaining an elevated $\dot{V}O_2$ for at least 2 h after a 5 h period of anoxia (Fig. 4a). Retention of metabolic end products and subsequent oxygen debt payment had been reported as characteristic of other species of freshwater pulmonates that were more intolerant of anoxia (von Brand and Mehlman, 1953; von Brand *et al.*, 1950). Similar oxygen debt payment has been recently reported for the intertidal prosobranchs *Littorina littorea* and *L. saxatilis* (McMahon and Russell-Hunter, 1978). Like *M. bidentatus*, these two species rarely experience hypoxic conditions in their more aerial high littoral environments, while low intertidal and subtidal gastropods (associated with aquatic environments in which hypoxic conditions are more likely to occur) generally do not display oxygen debt payment even after long periods of anoxia (McMahon and Russell-Hunter, 1974, 1978).

M. bidentatus does, however, display a short-term compensatory response to hypoxia marked by higher posthypoxic O_2 uptake rates at all O_2 tensions below air saturation, and by a distinct shift to stricter O_2 regulation of $\dot{V}O_2$ in posthypoxic individuals (Fig. 4b). Hence, posthypoxic individuals can maintain $\dot{V}O_2$ under concentrations as low as 30–60 torr (19%–38% of full air saturation with O_2) at levels equivalent to prehypoxic rates of O_2 uptake at full air saturation with O_2 (PO_2 = 160 torr). Such a compensatory response can allow posthypoxic individuals to maintain nearly normal levels of activity and metabolism during occasional short periods of hypoxia. Short-term hypoxic conditions can occur in both the shallow surface and subsurface waters of estuaries and salt marshes inhabited by *M. bidentatus*. Respiratory compensation for hypoxia of this type has been reported for a freshwater anyclid pulmonate, *Laevapex fuscus* (McMahon, 1972, 1973), an intertidal bivalve, *Mytilus edulis* (Bayne, 1975), several intertidal and subtidal prosobranchs (McMahon and Russel-Hunter, 1978), and most recently for a primitive estuarine pulmonate, *Amphibola crenata* (Shumway, 1981). *M. bidentatus* and *A. crenata* are amphibious air-breathers, in contrast to the more aquatic species of intertidal gastropods showing such compensatory responses to hypoxia (McMahon and Russell-Hunter, 1978). However, both of these species can commonly encounter hypoxic conditions when burrowing into the substratum for overwintering, or to avoid high tides, desiccation, or high temperatures (Hausman, 1932; Apley, 1967, 1970; Price, 1976, 1980; Watters, 1964; Shumway, 1981).

A. crenata appears to be relatively more aquatic than *M. bidentatus*; it is submerged on every high tide. It is able to maintain equivalent levels of $\dot{V}O_2$ in both air and water and displays a greater degree of O_2 regulation and respiratory compensation to hypoxia (Shumway, 1981) than does *M. bidentatus*. However, the ability to compensate for hypoxia may be of greatest advantage to the latter species during overwintering periods, when its encounters with low ambient oxygen concentrations may be more frequent.

Freshly taken from the field population, individuals of *M. bidentatus* have a clear circadian rhythm of aerial oxygen consumption. $\dot{V}O_2$ remains above average during dark evening hours (2000–0900 h) and below average during daylight hours (0900–2000 h) with maximum values occurring near 2359 h and minimum values near 1200 h (Fig. 5). There were no discernable tidal effects on the respiratory rhythms of *M. bidentatus* (Fig. 5). Similar circadian components in respiratory rhythms and activity (with maximum values at night or early morning and minimum values during the mid or late afternoon) have been described for high salt-marsh carbs of the genus *Uca* (Brown *et al.*, 1954, 1956; Bennett *et al.*, 1957) and for the intertidal snails *Nassarius obsoletus, Littorina littorea,* and *Urosalpinx cinereus* (Brown *et al.*, 1958; Sandeen *et al.*, 1954). All these more aquatic species also have a persistent tidal or circatidal rhythm of activity and metabolic rate not detected in *M. bidentatus*.

In its high salt-marsh habitat, *M. bidentatus* can remain for 4–8 days without tidal submergence during each semilunar period between spring tides (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1979, 1980). As do other extremely high littoral species, it has highly pronounced circadian (and weaker circatidal rhythms) in its activity and metabolic rates. In contrast, species from lower littoral environments, experiencing both tidal inundations and aerial exposure on a daily basis, tend to have both circadian and circatidal metabolic and activity rhythms (Palmer, 1974, 1976; Naylor, 1976; and references therein).

The pronounced circadian respiratory rhythm of *M. bidentatus* corresponds closely with reports of its field behavior. Apley (1967, 1970) states that *M. bidentatus* is inactive during periods of high mid-day ambient temperature. Cryptic behavior patterns allow *Melampus* to avoid desiccating conditions and to seek moist microhabitats. Summer-conditioned individuals of *M. bidentatus* are highly tolerant of short-term exposures to temperatures as great as 44–46°C (this study). However, *Melampus* is an extremely poor regulator of body water, losing approximately 25%–60% of total body water after 20 h exposure to relative humidities of 0%–95% (Russell-Hunter and Meadows, 1965; Price, 1976, 1980). Individuals of *M. bidentatus* undergoing desiccation have been shown to aggregate under both field and laboratory conditions, a behavior pattern which reduces the rate of water loss by about 25% (Price, 1980).

M. bidentatus is most active during the evening and early morning hours (Hausman, 1932) when ambient air temperature is at a minimum and relative humidity is at a maximum. This activity pattern corresponds directly with the circadian rhythm of $\dot{V}O_2$ recorded in this study. Also corresponding to increased activity during dark hours is the habit of carrying out 90% of egg-laying between 2359 h and 0800 h in both field populations and laboratory-held specimens (Russell-Hunter *et al.*, 1972). The reversal of metabolic rhythms in laboratory-maintained individuals (see Results and Fig. 5) may indicate that daily fluctuations of field ambient temperature and relative humidity are more important than light:dark cycles in entraining respiratory rhythms in *Melampus bidentatus*.

However, the light-dark cycle in *M. bidentatus* is important in timing the seasonal reproductive cycle with ratios greater than 14L:10D (and temperatures greater than 15°C) required for the initiation of copulation and oviposition (Apley, 1967; Price, 1976, 1979). A strict semilunar periodicity also is associated with egg-laying. Aggregation, copulation, and oviposition occur at successive new and full moons associated with peak spring tides during the annual reproductive period, which extends from late May through early July in the Little Sippewisset marsh (Russell-Hunter *et al.*, 1972). Semilunar periodicity of reproduction is retained by specimens held in the laboratory under constant conditions (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1979). (Seasonal and semilunar cycles of neurosecretion associated with the reproductive cycle have also been described for *M. bidentatus* [Price, 1976, 1979].)

In its natural history, ecology, and physiology, *Melampus bidentatus* is remarkably well adapted to life in the high littoral salt-marsh environment. Retaining a planktonic veliger along with an extremely high numerical fecundity (with obvious advantages to dispersal), it has evolved an elaborate semilunar pattern of reproduction and development that allows transport both of newly hatched veligers from, and of metamorphosing individuals back into, the upper reaches of the salt-marsh (Russell-Hunter *et al.*, 1972). During these events, there is a remarkable heterostrophic change in the growth patterns of the shell and mantle edge (Russell-Hunter *et al.*, 1972). They change from production of a low spired, nearly planospiral, sinistrally coiled, inflated shell (associated with the pelagic habit of the veliger) to generation of a truly turbinate, dextrally coiled shell with a narrower aperture in the metamorphosed spat. This second pattern is adapted to crawling on the marsh surface, desiccation resistance, and predator protection for the adult.

To this list of adaptations in *M. bidentatus* we can now add: (a) an extremely high thermal tolerance in both air and water as an adaptation to the temperature variation at the salt-marsh surface; (b) a high tolerance of submergence; (c) tolerance of varying salinities with little detectable effects of salinity on aquatic O_2 uptake rates; (d) a highly efficient aerial gas exchange at three to five times the rate maintained in water based upon the mantle-cavity diffusion lung and loss of the gill; (e) regulation of $\dot{V}O_2$ over the ambient temperature range allowing maintenance of a relatively efficient metabolic rates in an environment characterized by a high degree of temperature instability; (f) reverse acclimation of O_2 consumption rate when cold-conditioned as an adaptation that lowers O_2 demand and conserves energy stores in overwintering individuals (inactive-nonfeeding) entering hypoxic conditions in the salt-marsh substratum; (g) ability to compensate aquatic O_2 uptake rates to short-term exposures to hypoxia that allows near normal levels of $\dot{V}O_2$ to be maintained at O_2 tensions as low as 30–60 torr as can periodically occur in salt-marsh surface and subsurface waters; and (h) a clear circadian rhythm entrained to temperature and relative humidity variations (activity and metabolic rate are at a maximum during the dark hours of the day when desiccating conditions are minimal).

Morton (1955) describes the Ellobiidae, of which *Melampus bidentatus* is a member, as the most primitive pulmonate family, whose high littoral habitats foreshadow the purely terrestrial existence of the advanced stylommatophorans. *M. bidentatus* appears to lie very close to the terrestrial end of the gradient of evolved morphological, reproductive, and physiological characteristics associated with life in the intertidal environment. The higher pulmonates (Stylommatophora and Basommatophora) were presumably similarly preadapted for successful non-marine life (Russell-Hunter, 1979). In *M. bidentatus* the high temperature tolerance, high tolerance of desiccation, efficient aerial mode of oxygen consumption, temperature regulation of O_2 uptake, ability to enter extended overwintering periods of torpor associated with reverse respiratory acclimation to temperature, and marked circadian periodicity of metabolism and activity, are all adaptations closely associated with the terrestrial schesis of stylommatophoran pulmonates (see Peake, 1978; Ghiretti and Ghiretti-Magaldi, 1975; Machin, 1975; Boer and Joosse, 1975; and Duncan, 1975, for discussion of such adaptations in terrestial pulmonates).

Along with these more characteristically "terrestrial" adaptations, *M. bidentatus* retains a high tolerance of submergence (including both ability to withstand long periods of purely cutaneous aquatic gas exchange, and a high degree of salinity tolerance), relatively poor control of body water loss, a free-swimming pelagic veliger, and a semilunar reproductive cycle. These latter features are more closely associated with a littoral environment periodically and predictably inundated by high spring tides. Of these more aquatic features, retention by *M. bidentatus* of a planktonic veliger (and of the semiunar egg-laying cycle associated with the flooding of its high salt-marsh habitat by spring tides) appears to be by far the most important limiting factor that prevents this species from evolving a more terrestrial mode of life.

Of the approximately 22 genera of Ellobiids, only two, *Carychium* and *Zospeum*, have evolved a totally terrestrial mode of existence (Morton, 1955; Hubendick, 1978). *Carcychium tridentatum* occurs far inland, in deciduous forests but, like *M. bidentatus*, is cryptic and limited to very humid, moist microenvironments within damp forest floor leaf litter (Boycott, 1934; Watson and Verdcourt, 1953). *Zospeum* is limited to moist cave habitats in Europe (Hubendick, 1978). The ability of *Carychium* and *Zospeum* to lay tough, leathery terrestrial eggs in which larval stages are suppressed and from which hatch miniature adults ready to take up life in a terrestrial habitat, has allowed them (and, by inference, all of the higher stylommatophoran pulmonates) to escape the intertidal habitat to which the vast majority of ellobiid genera are presently restricted, and to evolve a completely terrestrial mode of life. This implies that selection pressures characteristic of life on the high littoral salt-marsh surface must account for the evolutionary origins of most of the physiological adaptations (specifically reported here for *M. bidentatus*) that are required for a purely terrestrial habit. Subsequent invasion of moist terrestrial enviornments from the high littoral required only the additional evolution of a terrestrial reproductive schesis involving larger, nearly cleidoic, eggs.

LITERATURE CITED

Apley, M. L. 1967. Field and experimental studies on pattern and control of reproduction in *Melampus bidentatus* (Say). Ph.D. dissertation, Syracuse University. 154 pp.

Apley, M. L. 1970. Field studies on life history, gonadal cycle and reproductive periodicity in *Melampus bidentatus* (Pulmonata: Ellobiidae). *Malacologia* **10:** 381–397.

Barnes, H., M. Barnes, and D. M. Finlayson. 1963. The seasonal changes in body weight, biochemical composition, and oxygen uptake of two common arctic cirripedes, *Balanus balanoides* and *B. balanus. J. Mar. Biol. Assoc. U. K.* **43:** 185–211.

Bayne, B. L. 1975. Aspects of the physiological condition in *Mytilus edulis* L., with special reference to the effects of oxygen tension and salinity. Pp. 213–238 in H. Barnes, Ed., *Proceedings of the ninth European marine biology symposium*, Aberdeen University Press, Aberdeen.

Bennett, M. F., J. Shriner, and R. A. Brown. 1957. Persistent tidal cycles of spontaneous motor activity in the fiddler crab, *Uca pugnax. Biol. Bull.* **112:** 267–275.

Berg, K. 1951. On the respiration of some molluscs from running and stagnant water. *Annee Biol.* **55:** 561–563.

Berg, K. 1952. On the oxygen consumption of Ancylidae (Gastropoda) from an ecological point of view. *Hydrobiologia* **5:** 225–267.

Berg, K. 1953. The problem of respiratory acclimation. *Hydrobiologia* **6:** 331–350.

Berg, K., J. Lumbye, and K. W. Ockelmann. 1958. Seasonal and experimental variations of the oxygen comsumption of the limpet *Ancylus fluviatilis. J. Exp. Biol.* **35:** 43–73.

BLAZKA, P. 1954. Temperaturadaptation des gesamtmetabolis mus bei der weinbergschnecke *Helix pomatia L. Zool. Jahr. Abt. Allg. Zool. Physiol.* **65:** 130–138.

BOER, H. H., AND J. JOOSSE. 1975. Endocrinology. Pp. 245–307 in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 1, Functional anatomy and physiology.* Academic Press, London.

BOYCOTT, A. E. 1934. The habitats of land mollusca in Britain. *J. Ecol.,* **22:** 1–388.

VON BRAND, T. 1946. Anaerobiosis in invertebrates. *Biodynamica Monographs* **4:** 1–328.

VON BRAND, T., H. D. BAERNSTEIN, AND B. MEHLMAN. 1950. Studies on the anaerobic metabolism and anaerobic carbohydrate consumption of some freshwater snails. *Biol. Bull.* **98:** 266–276.

VON BRAND, T., AND B. MEHLMAN. 1953. Relations between pre- and post anaerobic oxygen consumption and oxygen tension in some freshwater snails. *Biol. Bull.* **104:** 301–312.

BROEKHUYSEN, G. J. 1940. A preliminary investigation of the importance of desiccation, temperature, and salinity as factors controlling the vertical distribution of certain intertidal marine gastropods in False Bay, South Africa. *Trans. R. Soc. S. Afr.* **28:** 255–291.

BROWN, F. A., JR., M. F. BENNETT, AND H. M. WEBB. 1954. Persistent daily and tidal rhythms of O_2-consumption in fiddler crabs. *J. Cell. Comp. Physiol.* **44:** 477–506.

BROWN, F. A., JR., W. J. BRETT, AND H. M. WEBB. 1958. The rhythmic nature of metabolism in *Ilyanassa* in constant conditions. *Biol. Bull.* **115:** 345.

BROWN, F. A., JR., R. A. BROWN, H. M. WEBB, M. BENNETT, AND J. SHRINER. 1956. A persistant tidal rhythm of locomotor activity in *Uca pugnax. Anat. Rec.* **125:** 613–614.

BURKY, A. J. 1969. Biomass turnover, energy balance, and interpopulation variation in the stream limpet, *Ferrissia rivularis* (Say), with special reference to respiration, growth, and fecundity. Ph.D. dissertation, Syracuse University. 104 pp.

BURKY, A. J. 1970. Reverse acclimation at low temperature in the stream limpet, *Ferrissia rivularis* (Say). *Acta Cient. Venez.* **21:** 28.

BURKY, A. J. 1971. Biomass turnover, respiration, and interpopulation variation in the stream limpet, *Ferrissia rivularis* (Say). *Ecol. Monogr.* **41:** 235–251.

BURKY, A. J. 1977. Respiration chambers for measuring oxygen consumption of small aquatic molluscs with Clark-type polarographic electrodes. *Malacol. Rev.* **10:** 71–72.

BURKY, A. J., AND K. A. BURKY. 1976. Seasonal respiratory variation and acclimation in the pea clam, *Pisidium walkeri* Sterki. *Comp. Biochem. Physiol.* **55A:** 109–114.

CALOW, P., 1975. The respiratory strategies of two species of freshwater gastropods (*Ancylus fluviatilis* Müll and *Planorbis contortus* Linn) in relation to temperature, oxygen concentration, body size, and season. *Psyiol. Zoöl.* **48:** 114–129.

CAVENAUGH, G. M. 1956. *Formulae and Methods VI of the Marine Biological Laboratory Chemical Room.* Marine Biological Laboratory, Woods Hole, Massachusetts. Pp. 62–69.

DAVIES, P. S. 1965. Environmental acclimation in the limpet, *Patella vulgata* L. *Nature* **205:** 924.

DUNCAN, A. 1966. The oxygen consumption of *Potamopyrgus jenkinsi* (Smith) (Prosobranchiata) in different temperatures and salinities. *Verh. int. Ver. Limnol.* **16:** 1739–1751.

DUNCAN, A., AND R. Z. KLEKOWSKI. 1967. The influence of salinity on the survival, respiratory rate and heart beat of young *Potamopyrgus jenkinsi* (Smith) Prosobranchiata. *Comp. Biochem. Physiol.* **22:** 495–505.

DUNCAN, C. J. 1975. Reproduction. Pp. 309–365 in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 1, Functional anatomy and physiology.* Academic Press, London.

EVANS, R. G. 1948. The lethal temperatures of some common British littoral molluscs. *J. Anim. Ecol.* **17:** 165–173.

FARNIE, W. C. 1919. The structure of *Amphibola crenata* (Martyn). *Trans. Proc. N. Z. Inst.* **51:** 69–85.

FISCHER, P.-H., M. DUVAL, AND A. RAFFY. 1933. Études sur les échanges respiratoires des littorines. *Arch. Zool. Exp. Gen.,* **74:** 627–634.

FRAENKEL, G. 1961. Resistance to high temperatures in a Mediterranean snail, *Littorina neritoides. Ecology* **42:** 604–606.

FRAENKEL, G. 1966. The heat resistance of intertidal snails at Shirahama, Wakayama-ken, Japan. *Publ. Seto. Mar. Biol. Lab.* **14:** 185–195.

FRAENKEL, G. 1968. The heat resistance of intertidal snails at Bimini, Bahamas; Ocean Springs, Mississippi; and Woods Hole, Massachusetts. *Physiol. Zoöl.* **41:** 1–13.

GHIRETTI, F., AND A. GHIRETTI-MAGALDI. 1975. Respiration. Pp. 33–52 in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 1, Functional Anatomy and Physiology.* Academic Press, London.

GODDARD, C. K., AND A. W. MARTIN. 1966. Carbohydrate metabolism. Pp. 275–308 in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Mollusca, Vol. II.* Academic Press, London.

GOWANLOCH, J. N., AND F. R. HAYES. 1926. Contributions to the study of marine gastropods. I. The physical factors, behavior, and intertidal life of *Littorina. Contrib. Can. Biol. Fish.* **3:** 133–165.

Hilbish, T. J. 1981. Latitudinal variation in freezing tolerance of *Melampus bidentatus* (Say) (Gastropoda: Pulmonata). *J. Exp. Mar. Biol. Ecol.* **52:** 283–297.

Hausman, S. A. 1932. A contribution to the ecology of the salt marsh snail, *Melampus bidentatus* Say. *Am. Nat.* **66:** 541–545.

Hubendick, B. 1978. Systematics and comparative morphology of the Basommatophora. Pp. 1–47, in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 2A, Systematics, evolution and ecology.* Academic Press, London.

Kinné, O. 1964. The effects of temperature and salinity on marine and brackish water animals II. Salinity and temperature salinity combinations. *Oceanogr. Mar. Biol. Ann. Rev.* **2:** 281–339.

Kinné, O. 1971. Salinity—invertebrates. Pp. 821–995, in O. Kinné, Ed., *Marine ecology, Vol. I.* Wiley-Interscience, New York.

Krog, H. 1954. Temperature sensitivity of freshwater gammarids. *Biol. Bull.* **107:** 397–410.

Lewis, J. B. 1963. Environmental and tissue temperatures of some tropical intertidal marine animals. *Biol. Bull.* **124:** 277–284.

Lockwood, A. P. M. 1976. Physiological adaptation to life in estuaries. Pp. 315–392 in R. C. Newell, Ed., *Adaptation to Environment: Essays on the Physiology of Marine Animals.* Butterworth and Co., Ltd., London.

Lumbye, J. 1958. The oxygen consumption of *Theodoxus fluviatilis* (L.) and *Potamopyrgus jenkinsi* (Smith) in brakish and fresh water. *Hydrobiologia* **10:** 245–262.

Lumbye, J., and L. E. Lumbye. 1965. The oxygen consumption of *Potamopyrgus jenkinsi* (Smith). *Hydrobiologia* **25:** 489–500.

Machin, J. 1975. Water relationships. Pp. 105–163, in V. Fretter and J. Peake, Eds., *Pulmonates, Vol.* 1, *Functional Anatomy and Physiology.* Academic Press, London.

Mangum, C. P., and W. Van Winkle. 1973. Responses of aquatic invertebrates to declining oxygen conditions. *Am. Zool.* **13:** 529–541.

McMahon, R. F. 1972. Interpopulation variation and respiratory acclimation in the bioenergetics of *Laevapex fuscus.* Ph.D. dissertation, Syracuse University. 164 pp.

McMahon, R. F. 1973. Respiratory variation and acclimation in the freshwater limpet, *Laevapex fuscus. Biol. Bull.* **145:** 492–508.

McMahon, R. F. 1975. Growth, reproduction and bioenergetic variation in three natural populations of a freshwater limpet *Laevapex fuscus* (C. B. Adams). *Proc. Malacol. Soc. Lond.* **41:** 331–351.

McMahon, R. F. 1976. Effluent-induced interpopulation variation in the thermal tolerance of *Physa virgata* Gould. *Comp. Biochem. Physiol.* **55A:** 23–28.

McMahon, R. F., and B. S. Payne. 1980. Variation of thermal tolerance limits in populations of *Physa virgata* Gould (Mollusca: Pulmonata). *Am. Midl. Nat.* **103:** 218–230.

McMahon, R. F., and W. D. Russell-Hunter. 1974. Responses to low oxygen stress in relation to the ecology of littoral and sub-littoral snails. *Biol. Bull.* **147:** 490.

McMahon, R. F., and W. D. Russell-Hunter. 1977. Temperature relations of aerial and aquatic respiration in six littoral snails in relation to their vertical zonation. *Biol. Bull.* **152:** 182–198.

McMahon, R. F., and W. D. Russell-Hunter. 1978. Respiratory responses to low oxygen stress in marine littoral and sublittoral snails. *Physiol. Zoöl.* **51:** 408–424.

Morton, J. E. 1955. The evolution of the Ellobiidae with a discussion of the origin of the pulmonata. *Proc. Zool. Soc. Lond.* **125:** 127–168.

Naylor, E. 1976. Rhythmic behavior and reproduction in marine animals. Pp. 393–429 in R. C. Newell, Ed., *Adaptation to environment: Essays on the physiology of marine animals.* Butterworth and Co., Ltd., London.

Newell, R. C. 1973. Factors affecting the respiration of intertidal invertebrates. *Am. Zool.* **13:** 513–528.

Newell, R. C. 1976. Adaptations to intertidal life. Pp. 1–82 in R. C. Newell, Ed., Adaptation to environment: Essays on the physiology of marine animals. Butterworth and Co., Ltd., London.

Newell, R. C. 1979. *Biology of intertidal animals,* Third edition. Marine Ecological Survey Ltd., Faversham, Kent, U. K.. 781 pp.

Newell, R. C., and V. I. Pye. 1970a. Seasonal changes in the effect of temperature on the oxygen consumption of the winkle *Littorina littorea* (L.) and the mussell *Mytilus edulis* L. *Comp. Biochem. Physiol.* **34:** 367–383.

Newell, R. C. and V. I. Pye. 1970b. The influence of thermal acclimation on the relation between oxygen consumption and temperature in *Littorina littorea* (L.) and *Mytilus edulis* L. *Comp. Biochem. Physiol.* **34:** 385–397.

Newell, R. C., and V. I. Pye. 1971a. Quantitative aspects of the relationship between metabolism and temperature in the winkle *Littorina littorea* (L.). *Comp. Biochem. Physiol.* **38B:** 635–650.

NEWELL, R. C., AND V. I. PYE. 1971b. Temperature induced variations in the respiration of mitochondria from the winkle *Littorina littorea* (L.). *Comp. Biochem. Physiol.* **40B:** 249–261.

NEWELL, R. C., AND A. ROY. 1973. A statistical model relating the oxygen consumption of a mollusc (*Littorina littorea*) to activity, body size, and environmental conditions. *Physiol. Zoöl.* **46:** 253–275.

PACKARD, G. C. 1972. Inverse compensation for temperature in oxygen consumption of the hylid frog *Pseudacris triseriata. Physiol Zoöl.* **45:** 270–275.

PALMER, J. D. 1974. *Biological clocks in marine organisms: The control of physiological and behavioral tidal rhythms.* John Wiley and Sons, New York. 173 pp.

PALMER, J. D. 1976. *An introduction to biological rhythms.* Academic Press, London. 375 pp.

PARRY, G. D. 1978. Effects of growth and temperature acclimation on metabolic rate in the limpet, *Cellana tramoserica* (Gastropoda: Patellidae). *J. Anim. Ecol.* **47:** 351–368.

PEAKE, J. 1978. Distribution and ecology of the stylommatophora. Pp. 429–526 in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 2A. Systematics, evolution and ecology.* Academic Press, London.

PRICE, C. H. 1976. Behavior, physiological ecology, and neurosecretion in the reproduction and water relations of *Melampus bidentatus* Say (Mollusca: Pulmonata). Ph.D. dissertation, Syracuse University, 172 pp.

PRICE, C. H. 1979. Physical factors and neurosecretion in the control of reproduction in *Melampus* (Mollusca: Pulmonata). *J. Exp. Zool.* **207:** 269–282.

PRICE, C. H. 1980. Water relations and physiological ecology of the salt marsh snail, *Melampus bidentatus* Say. *J. Exp. Mar. Biol. Ecol.* **45:** 51–67.

PROSSER, C. L. 1973. Temperature. Pp. 362–428 in C. L. Prosser, Ed., *Comparative animal physiology,* Third ed. W. B. Saunders Company, Philadelphia.

RAFFY, A. 1933. Recherches sur le métabolisme respiratorie des poikilothermes aquatiques. *Ann. Inst. Océanogr. Monaco* **13:** 263–396.

ROBERTS, J. L. 1960. The influence of photoperiod on thermal acclimation. *Zool. Anz.* **24:** 73–78.

ROBERTS, J. L. 1966. Systematic versus cellular acclimation to temperature by poikilotherms. *Helgolaender Wiss. Meeresunters.* **14:** 451–465.

RUSSELL-HUNTER, W. D. 1979. *A life of invertebrates.* Macmillan Publishing Company, New York. 650 pp.

RUSSELL-HUNTER, W. D., M. L. APLEY, AND R. D. HUNTER. 1972. Early life-history of *Melampus* and the significance of semilunar synchrony. *Biol. Bull.* **143:** 623–656.

RUSSELL-HUNTER, W. D., AND A. G. EVERSOLE. 1976. Evidence for tissue degrowth in starved freshwater pulmonate snails (*Helisoma trivolvis*) from tissue, carbon and nitrogen analysis. *Comp. Biochem. Physiol.* **54A:** 447–453.

RUSSELL-HUNTER, W. D., AND R. L. MEADOWS. 1965. Aspects of water physiology in the salt-marsh pulmonate snail, *Melampus bidentatus. Biol. Bull.* **129:** 409.

SANDEEN, M. I., C. G. STEPHENS, AND F. A. BROWN, JR. 1954. Persistent daily and tidal rhythms of oxygen consumption in two species of marine snails. *Physiol. Zoöl.* **27:** 350–356.

SANDISON, E. E. 1967. Respiratory response to temperature and temperature tolerance of some intertidal gastropods. *J. Exp. Mar. Biol. Ecol.* **1:** 271–281.

SCHLIEPER, C. 1971. Physiology of brackish water. Part II. Pp. 211–250 in A. Remane, and C. Schlieper (Authors), *Biology of brackish water.* John Wiley and Sons, Inc., New York.

SHIRLEY, T. C., G. Y. DENOUX, AND W. B. STICKLE. 1978. Seasonal respiration in the marsh periwinkle, *Littorina irrorata. Biol. Bull.* **154:** 322–334.

SHUMWAY, S. E. 1981. Factors effecting oxygen consumption in the marine pulmonate *Amphibola crenata* (Gmelin, 1791). *Biol. Bull.* **159:** 332–347.

TOULMOND, M. A. 1967a. Étude de la consommation d'oxygène en fonction du poids, dans l'air et dans l'eau, chez quatre espèces du genre *Littorina* (Gastropoda, Prosobranchiata). *C. R. Hebd. Seances Acad. Sci. Paris* **264:** 636–638.

TOULMOND, A. 1967b. Consommation d'oxygène, dans l'air et dans l'eau, chez quatre gastéropodes du genre *Littorina. J. Physiol.* (*Paris*) **59:** 303–304.

WATSON, H., AND B. VERDCOURT. 1953. The two British species of *Carychium. J. Conchol,* **23:** 306–324.

WATTERS, P. A. 1964. Distribution of *Amphibola crenata* (Pulmonata) in the Dunedin area, with notes on the probable origin of the species. *Trans. R. Soc. New Zealand,* **4:** 117–134.

DE ZWAAN, A., AND T. C. M. WIJSMAN. 1976. Anaerobic metabolism in Bivalvia (Mollusca). Characteristics of anaerobic metabolism. *Comp. Biochem. Physiol.* **54B:** 313–324.

Reference: *Biol. Bull.* **161**:270-280. (October, 1981)

MORPHOLOGICAL ORGANIZATION OF CRUSTACEAN PIGMENTARY EFFECTORS

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ABSTRACT

The basic morphological organization of pigmentary effectors from the epidermis and internal organs of the freshwater palaemonid shrimps *Macrobrachium olfersii* and *Macrobrachium heterochirus*, the marine shrimps *Palaemon affinis* and *Palaemon northropi*, and the fiddler crab *Uca rapax*, was investigated by light and transmission electron microscopy. All pigmentary effectors (chromatosomes) examined comprise groups of 2-12 tightly bound, uninucleate cells (chromatophores), each possessing one or two cell extensions. Constituent chromatophores contained either a single or several pigment granule types of one or different colors. Desmosomes were noted between the membranes of adjacent chromatophores, although a limiting basement membrane was only present surrounding chromatosomes from the internal organs. Multinucleate or syncytial chromatophores were not encountered. Terminology currently used to describe crustacean pigmentary units is discussed in relation to the different levels of organization revealed.

INTRODUCTION

Although crustacean chromatophores have long been the subject of physiological investigations (see Fingerman, 1963, 1965, 1969, 1970; Fingerman *et al.*, 1975; Lambert and Fingerman, 1978, 1979, for reviews and references) and, more recently, of ultrastructural studies (Elofsson and Kauri, 1971; Chassard-Bouchaud and Hubert, 1971a, b; Green and Neff, 1972; Green, 1973; Robison and Charlton, 1973; McNamara, 1979, 1980, 1981a), the basic morphology of these pigmentary effectors is still unclear and the terminology used to describe them needs revision.

Pouchet (1876) considered each crustacean chromatophore to be a single cell containing a single pigment. However, Keeble and Gamble (1904, p. 304) stated of *Macromysis flexuosa* that "The mature chromatophores are not single cells but are complex organs." Of decapod crustacean chromatophores, these authors said "Several pigments may occur in the centre of a single chromatophore, and the evidence goes to show that each pigment occupies one or more 'cells' and the processes of these cells, and that the chromatophores are clusters of such." Their general conclusion was that "The chromatophores are polynuclear stellate masses of cytoplasm, the central part of which is bounded by a distinct membrane."

Ballowitz (1914) proposed the term *chromatosome* to describe pigmentary effectors comprising groups of closely united, unicellular chromatophores. However, this concept has since been modified, for example, by Barrington (1964, p. 300): "Among invertebrates chromatophores are best known in Crustacea where they may form syncytial complexes called chromatosomes," and by Nicol (1967, p. 510): "Adaptive colour responses are a notable feature of the behaviour of many decapods. The chromatophores concerned in these changes are multinucleate or syncytial structures lying in the skin, or sometimes at deeper levels."

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Recent ultrastructural studies (Elofsson and Kauri, 1971; Hubert and Chassard-Bouchaud, 1976; McNamara, 1976, 1981b) confirmed Ballowitz' (1914) proposition that pigmentary effectors may be multicellular. Thus, chromatosomes contain separate chromatophores of similar or different colors, each color being represented by several cells. A basement membrane limiting the chromatosome has been described (Elofsson and Kauri, 1971).

To ascertain to what extent crustacean pigmentary effectors, including those exhibiting only a single color, are multicellular entities, the present study examines the basic morphological organization of the chromatic units of a variety of crustaceans including marine and freshwater palaemonid shrimps and a marine brachyuran crab. A suitable terminology is proposed to describe these effectors at all levels of organization.

Materials and Methods

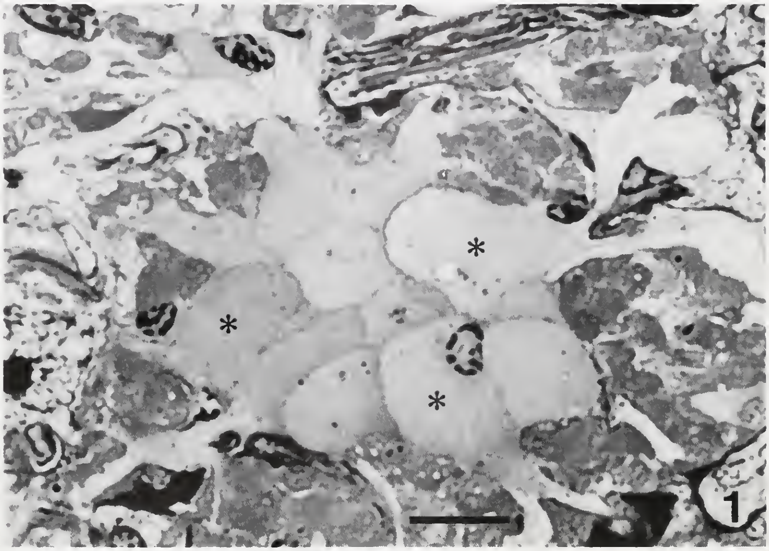
Specimens of the freshwater palaemonid shrimps *Macrobrachium heterochirus* and *M. olfersii*, and the fiddler crab *Uca rapax*, were collected from small streams discharging into the São Sebastião Channel at or near the Guaecá Beach (23°49′18″S; 45°27′18″W) in the State of São Paulo, Brazil. The marine palaemonids *Palaemon northropi* and *P. affinis* were collected from intertidal rock pools near the Guaecá Beach, Brazil, and from the Kaikoura Peninsula, New Zealand (42°25′S; 173°42′E), respectively.

For light microscopic and ultrastructural examination, small pieces of brachiostegite, ventral nerve cord, and arthrodial membrane, containing chromatophores, were dissected and prefixed in a 0.1 M, Na-cacodylate buffered, paraformaldehyde-glutaraldehyde (65 mM: 250 mM) solution also containing Ca (12 mM), K (5 mM) and Mg (3 mM) as chlorides, for 1.5 h at 4°C. The material was subsequently washed at 4°C for 15 min, in three changes of the above solution minus the paraformaldehyde-glutaraldehyde, postfixed in 0.1 M, Na-cacodylate buffered, 1% osmium tetroxide for 2 h at 4°C, dehydrated in a cold ethanol series, passed through propylene oxide, and embedded in either Araldite 6005 or Spurr's (1969) resin. Thick (0.3–0.5 μm) and thin (silver-gold) sections were cut with glass knives on either an LKB Bromma Ultratome III or a Porter-Blum Sorvall MT-2 ultramicrotome. Thick sections were stained on a hotplate with a solution comprising equal parts of 1% methylene and toluidine blue in 1% aqueous borax. They were then examined in Zeiss or Leitz Orthoplan photomicroscopes. Thin sections were stained with 2% aqueous uranyl acetate followed by Reynolds' (1963) lead citrate. They then were examined in Jeolco JEM 100B, Philips EM 301 or Zeiss EM9S-2 electron microscopes at accelerating voltages of 60 or 80 kV.

Measurements were taken directly from the micrographs and are given in the text as the mean value ± the standard error of the mean. The number of measurements used (n) is given in parentheses.

Results

All the pigmentary effectors examined, regardless of species or location, proved to be groups of intimately associated, uninucleate, pigment bearing cells. In the integumental epidermis of the freshwater shrimp *Macrobrachium olfersii* such cellular composites, or chromatosomes, attain great complexity (Fig. 1), comprising up to 12 uninucleate, monochromatic cells. Each chromatophore has a roughly spherical cell body (Fig. 2) of 25.0 ± 1.4 μm diameter ($n = 15$), from which project one, or at most two, cell extensions. At their junction with the cell body, these extensions, when filled with pigment, measure about 5 μm diameter, tapering to 1–2 μm diameter in their most distal regions. The entire chromatosome measures



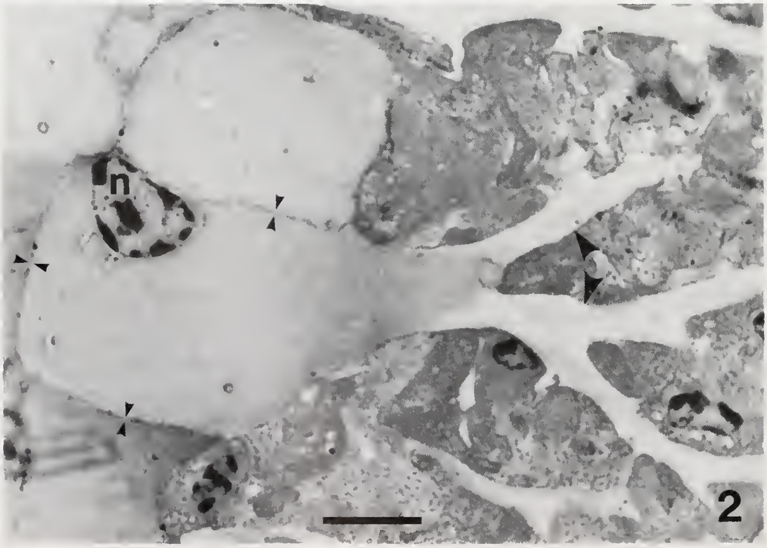


FIGURE 1. Light micrograph of longitudinal, thick section through red, epidermal chromatosome in *Macrobrachium olfersii*. Each component chromatophore (*) is distinct and uninucleate. Pigments are fully aggregated. Scale bar, 20 μm.

FIGURE 2. Light micrograph of longitudinal, thick section through a single, uninucleate chromatophore that was a part of red, epidermal chromatosome in *Macrobrachium olfersii*. Two cell extensions (arrows) derive from the cell body. Chromatophore boundaries (apposed arrows) are distinctly defined. Pigments are fully aggregated. Nucleus (n). Scale bar, 10 μm.

about 184.7 ± 12.4 μm diameter (n = 15), including the cell extensions. In these monochromatic, red chromatophores, the pigment granules are spherical to polymorphic, of 94.8 ± 3.7 nm diameter (n = 48), and lack a limiting membrane.

A very similar situation was revealed for the epidermal chromatosomes of the marine shrimp *Palaemon affinis* (Figs. 3, 4). The living chromatosomes of this shrimp contain at least three pigment colors. A peripherally located yellow pigment lies distally to a brown pigment, and large (approx. 1–2 μm diameter), iridescent blue, oblong granules are distributed throughout the chromatophore cell bodies and extensions. The yellow and brown pigments are not resolvable as granules at the light microscopic level in the living cells.

When seen in thin sections, the individual chromatophores composing these chromatosomes also contain at least three pigment granule types: One is a membrane-bounded polygon of 0.5–1.5 μm in the longest axis (the blue pigment crystal). An amembranous spherical granule of 140.0 ± 5.0 nm diameter (n = 150), encountered in the cell body, contains the brown pigment (Fig. 5). The yellow pigment, which appears as a similar, spherical granule of 80.0 ± 2.0 nm diameter (n = 250), is abundant in sections taken from the peripheral extremities of the chromatophore extensions. Both granule types can be seen mixed with the polygonal, membrane-limited pigment crystals. Such chromatophores are thus dichromatic, while the chromatosome is polychromatic.

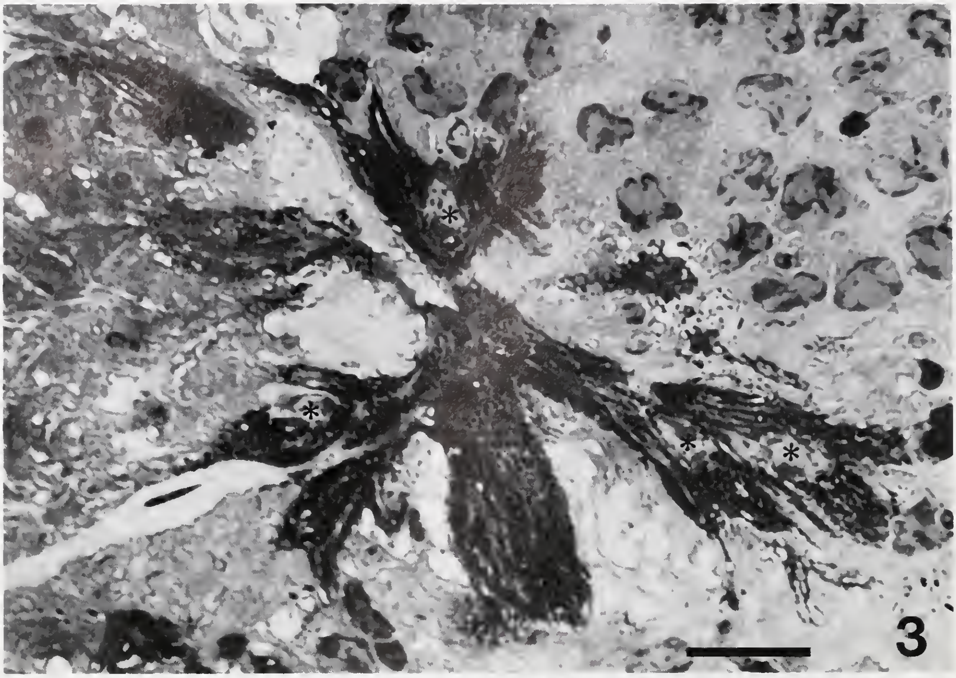
The brown chromatosomes enveloping the ventral nerve cord of the freshwater shrimp *Macrobrachium heterochirus*, although less complex than those of the epidermis, are small aggregates of 3–4 uninucleate chromatophores (Fig. 6). Component chromatophores, although exhibiting only a single color (brown), contain spherical, membrane-limited granules of 600.0 ± 50.0 nm diameter (n = 30) (Fig. 6), in addition to numerous spherical, amembranous granules of 71.4 ± 1.8 nm diameter (n = 30) (Fig. 7). The brown, ventral nerve cord chromatosomes of the marine shrimp *Palaemon northropi*, of similar basic organization, also contain two pigment granule types. One is a polygonal, membrane-limited granule of 400.0 ± 20.0 nm diameter (n = 24), which exhibits a paracrystalline substructure comprising alternating electron-dense and electron-lucent bands, repeating at 8.40 ± 0.13 nm intervals (Fig. 8). The other granule is spherical, of 51.1 ± 2.4 nm diameter (n = 20), and lacks a limiting membrane (Fig. 8).

In all the chromatosomes of the shrimp species examined, adjacent chromatophores are strongly bound to each other by well developed membrane junctions (Fig. 9). Only the ventral nerve cord chromatosomes are limited by a basement-membrane-like structure, probably composed of collagen fibres. Epidermal chromatosomes showed no evidence of a basement membrane, but exhibited more strongly developed areas of desmosomal contact.

The black epidermal chromatosomes of the brachyuran crab *Uca rapax* were the simplest encountered. These chromatosomes comprise only 2–3 chromatophores, each uninucleate (Fig. 10), containing a single type of highly electron dense, membrane-limited spherical pigment granule of 300.0 ± 10.0 nm diameter (n = 29). Small and infrequent membrane junctions bind the plasmalemmae of adjacent chromatophores within the chromatosome. A basement membrane was not noted.

DISCUSSION

Morphological data from the present study clearly reveal that pigmentary effectors from the epidermis and internal organs of both natant and brachyuran decapods are closely bound groups of several uninucleate chromatophores. Single



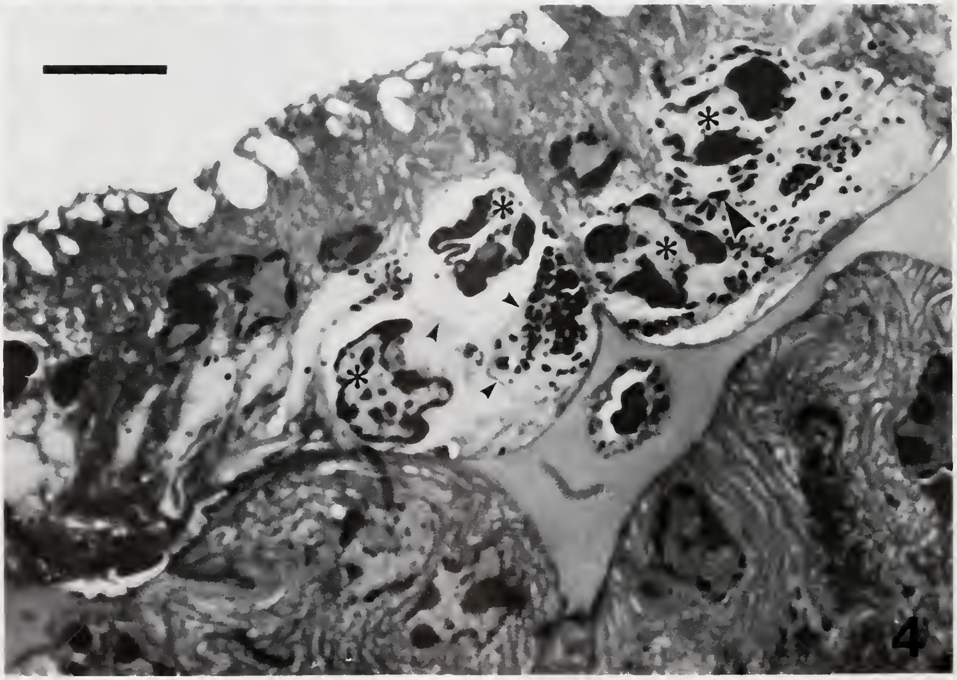


FIGURE 3. Light micrograph of longitudinal thick section through a polychromatic epidermal chromatosome in *Palaemon affinis*. Several chromatophores are present, each uninucleate. Pigments are fully dispersed. Nucleus (*). Scale bar, 20 μm.

FIGURE 4. Light micrograph of transverse section through polychromatic, epidermal chromatosome in *Palaemon affinis*. Several uninucleate chromatophores, separated by distinct boundaries (small arrows) are discernable. The darkly staining polyhedrons (large arrow) are blue pigment crystals; the lightly staining areas are brown pigment (*cf.*, Fig. 5). Pigments fully dispersed. Nucleus (*). Scale bar, 10 μm.

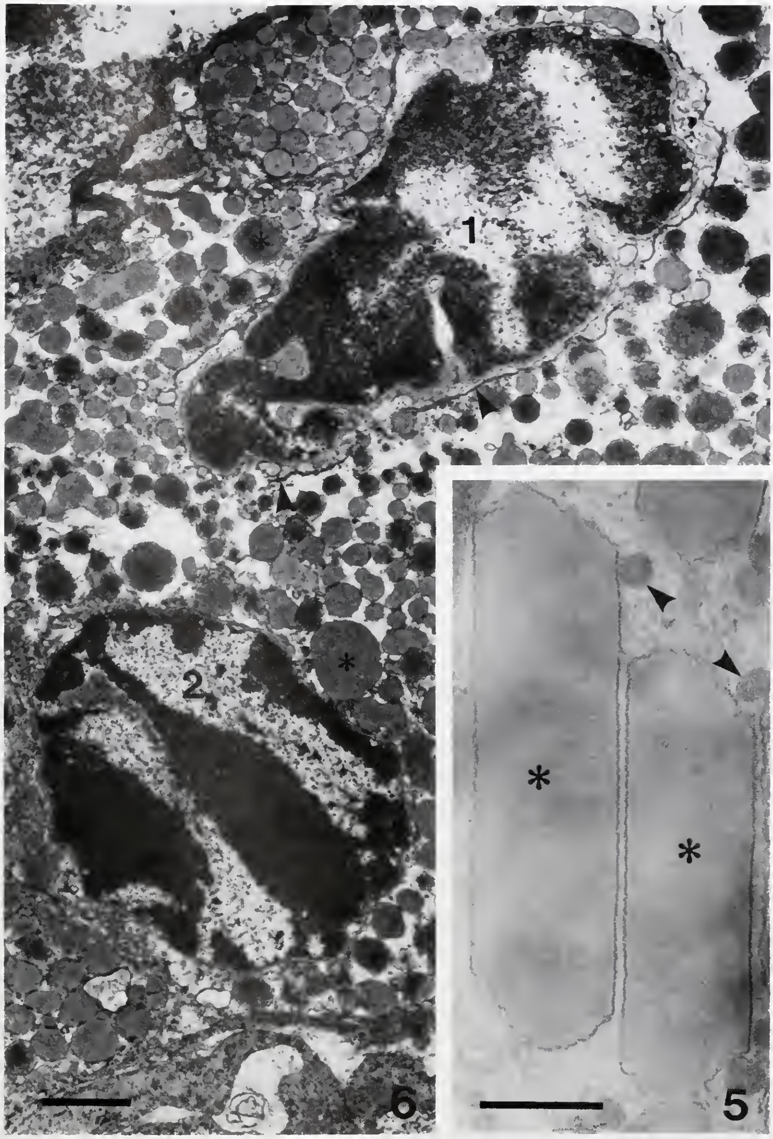
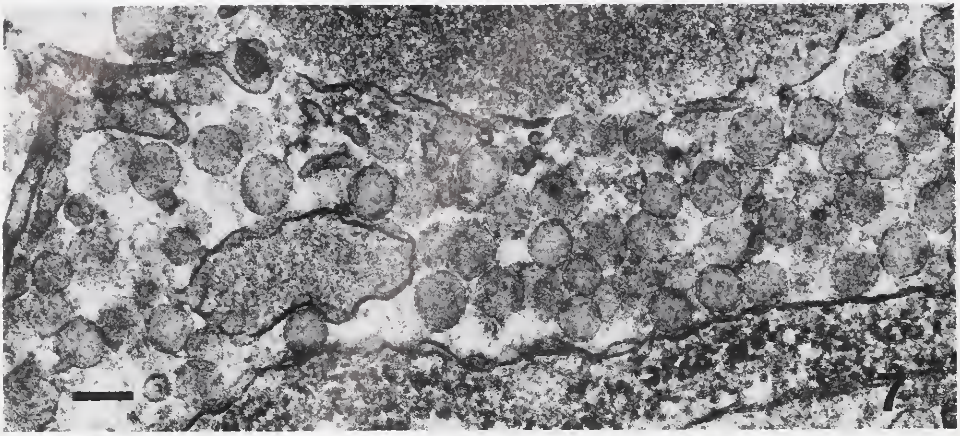


FIGURE 5. Membrane-bounded polyhedral pigment crystals (*) and amembranous spherical pigment granules (arrows) in dichromatic epidermal chromatophore from *Palaemon affinis*. Scale bar, 0.4 μm.

FIGURE 6. Transverse section through brown chromatosome from *Macrobrachium heterochirus* ventral nerve cord, revealing multicellular nature. Nuclei (1, 2) are separated by two closely apposed plasmalemmae (arrows). Note large, membrane-bounded spherical pigment granules (*). Scale bar, 1.5 μm.

chromatophores are rare. Each component chromatophore may contain a single type of pigment granule, *e.g.*, the monochromatic red epidermal chromatophores of *Macrobrachium olfersii* and the monochromatic black epidermal chromatophores of *Uca rapax* (*cf.* Green and Neff, 1972), or may contain several pigments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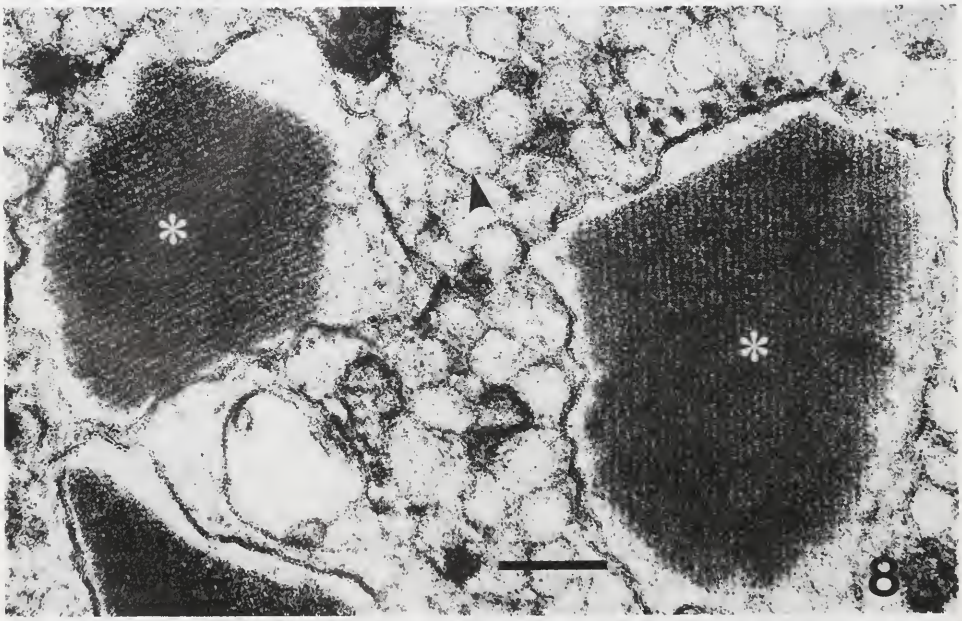
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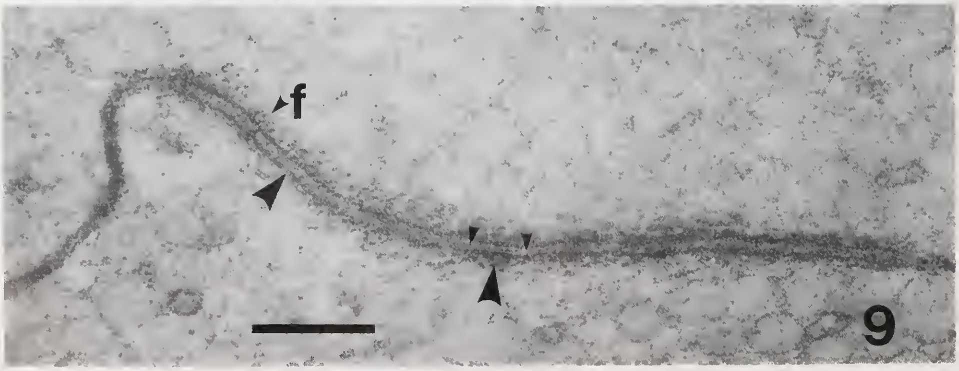
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of very different morphology and color, *e.g.*, the dichromatic, epidermal chromatophores of *Palaemon affinis*. Robison and Charlton (1973) found up to four different pigment granule types within a single ovarian chromatophore of *Palaemonetes vulgaris*, while Hubert and Chassard-Bouchaud (1976) reported two morphologically distinct granules in red, epidermal chromatophores of *Palaemon serratus*. McNamara (1981a) described three pigment granule types from the hind gut chromatophores of *Palaemon affinis*. In the above examples, the different pigment granules were the source of different pigment colors. However, in the brown chromatosomes of the ventral nerve cord of *Macrobrachium heterochirus* (and other *Macrobrachium* species; McNamara, 1981b) only a single color is visible even though two morphologically distinct pigment granules are present in the component chromatophores. Such chromatophores are, strictly, monochromatic. Thus, considered at the level of the smallest functional unit, the individual chromatophore may contain pigment of a single color (regardless of the number of granule types present), being therefore monochromatic, or may contain several pigments of different colors and morphologies, being di- or polychromatic.

Chromatosomes likewise differ considerably, particularly in the number of constituent chromatophores. They are probably simplest in the brachyuran crabs, comprising 2–3 cells, and most complex in the palaemonid shrimps, where up to 15 cells may be encountered. All the chromatophores composing the chromatosome may contain the same pigment (monochromatic chromatosome), *e.g.*, red, epidermal chromatosomes of *Macrobrachium olfersii* and black, epidermal chromatosomes of *Uca rapax*; or they may contain different pigments, each chromatophore bearing pigment of a single color (Elofsson and Kauri, 1971) (polychromatic chromatosome). The most complex situation, thus far noted only in palaemonid shrimps, *e.g., Palaemon affinis*, is that in which di- or polychromatic chromatophores containing, separately, brown and blue, yellow and blue, and red and blue pigments, are united to form polychromatic chromatosomes.

Neither chromatosomes nor chromatophores are multinucleate or syncytial; this idea probably arose as a misinterpretation of light microscopical studies using liver material. Parker (1948, pp. 54–55) discussed this aspect. His proposed terminology is satisfactory in light of the present study, although chromatophores proper are now known to contain more than one kind and color of pigment. Brown (1973, p. 917) stated that "the several pigments within a single chromatophore may show a considerable degree of independence of one another." This observation now may well be reinterpreted as reflecting the independence of pigment translocation within a single chromatophore type comprising the chromatosome. However, although the smallest functional unit of the crustacean pigmentary effector is the chromatophore, the chromatosome probably forms the basis of any chromatic arrangement.

The presence of a basment-membrane-like structure limiting the chromatosome (Elofsson and Kauri, 1971) was only verified for the chromatosomes situated on

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FIGURE 7. Amembranous spherical pigment granules from brown ventral-nerve-cord chromatophore in *Macrobrachium heterochirus*. Scale bar, 0.1 μm.

FIGURE 8. Paracrystalline membrane-limited pigment granules (*) and spherical amembranous pigment granules (arrow) in brown ventral-nerve-cord chromatophore of *Palaemon northropi*. Scale bar, 0.1 μm.

FIGURE 9. Desmosome (large arrows) formed between plasmalemmae of two adjacent chromatophores in red, epidermal chromatosome of *Macrobrachium olfersii*. Note fine electron-dense line (arrows) between the plasmalemmae, and the fibrous material (f) below each membrane. Scale bar, 0.2 μm.

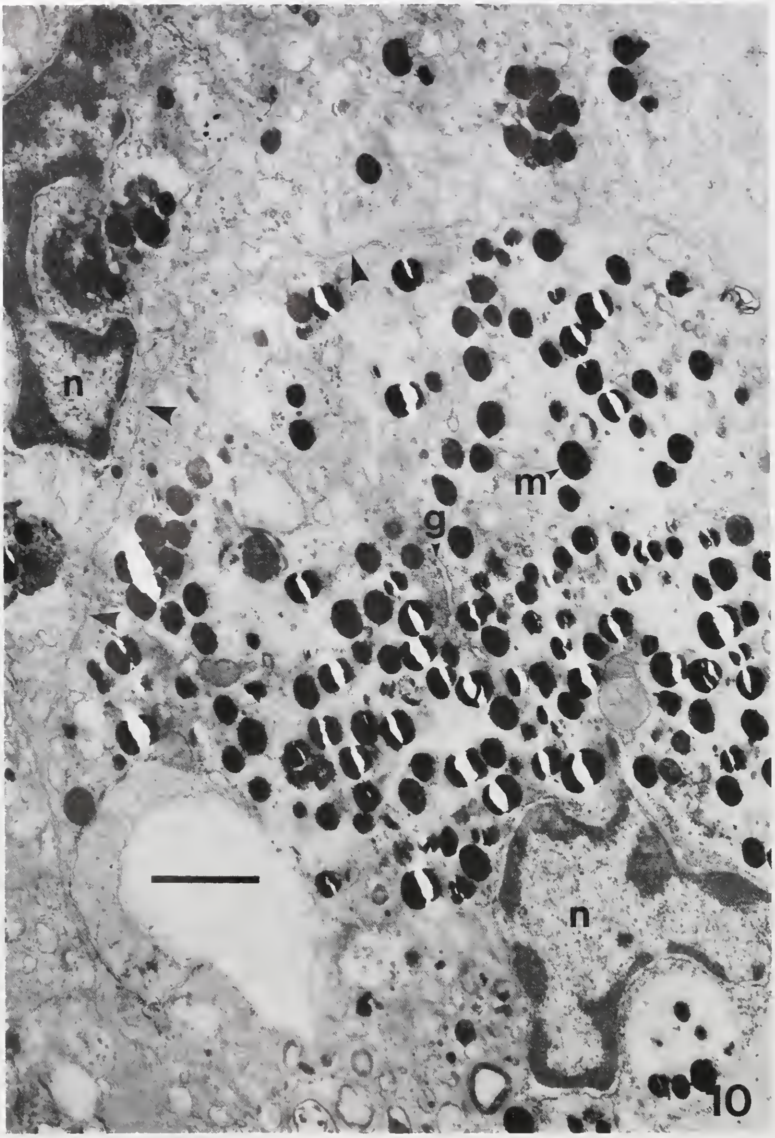

FIGURE 10. Longitudinal section through black, epidermal chromatosome in *Uca rapax*. Two clearly defined uninucleate chromatophores are discernable. Arrows indicate limiting membranes of each chromatophore. Granular endoplasmic reticulum (g), melanin granule (m), nucleus (n). Scale bar, 1 μm.

the internal organs, *e.g.*, ventral nerve cord and hind gut (McNamara, 1981a). Electron microscopical examination of epidermal chromatosomes in both shrimps and crab did not reveal such a structure. Desmosomes have not been previously demonstrated in crustacean chromatosomes.

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LITERATURE CITED

BALLOWITZ, E. 1914. Die chromatischen Organe, Melaniridosomen, in der Haut der Barsche (*Perca* und *Acerina*). *Z. Wiss. Zool.* **100:** 1-35.

BARRINGTON, E. J. 1964. Hormones and the control of color. Pp. 299-363 in G. Pincus, K. V. Thimann, E. B. Astwood, Eds, *The hormones,* Vol. IV. Academic Press, New York.

BROWN, F. A., JR. 1973. Chromatophores and color change. Pp. 915-950 in C. L. Prosser, Ed., *Comparative animal physiology,* 3rd Edition. W. B. Saunders Co., Philadelphia.

CHASSARD-BOUCHAUD, C., AND M. HUBERT. 1971a. Premières données relatives à la presence de microtubules cytoplamsiques dans les chromatophores de crustacés. *C. R. Acad. Sci. Paris* **272:** 1405-1408.

CHASSARD-BOUCHAUD, C., AND M. HUBERT. 1971b. Organisation scalariforme du réticulum endoplasmique dans les chromatophores de *Palaemon serratus* (Pennant) (Crustacé Décapode). *C. R. Acad. Sci. Paris* **273:** 1820-1823.

ELOFSSON, R., AND T. KAURI. 1971. The ultrastructure of the chromatophores of *Crangon* and *Pandalus. J. Ultrastruct. Res.* **36:** 263-270.

FINGERMAN, M. 1963. *The control of chromatophores.* Pergamon-Macmillan, New York. 184 pp.

FINGERMAN, M. 1965. Chromatophores. *Physiol. Rev.* **45:** 296-339.

FINGERMAN, M. 1969. Cellular aspects of the control of physiological color changes in crustaceans. *Am. Zool.* **9:** 443-452.

FINGERMAN, M. 1970. Comparative physiology: chromatophores. *Ann. Rev. Physiol.* **32:** 345-372.

FINGERMAN, M., S. W. FINGERMAN, AND D. T. LAMBERT. 1975. Colchicine, cytochalasin B, and pigment movements in ovarian and integumentary erythrophores of the prawn, *Palaemonetes vulgaris. Biol. Bull.* **149:** 165-177.

GREEN, J. P., AND M. R. NEFF. 1972. A survey of the fine structure of the integument of the fiddler crab. *Tiss. Cell* **4:** 137-171.

GREEN, J. P. 1973. Aspects of the pigmentary system of the decapod crustacean *Uca pugnax. Malaysian J. Sci.* **2A:** 29-38.

HUBERT, M., AND C. CHASSARD-BOUCHAUD. 1976. Étude ultrastructurale des chromatophores de *Palāemon serratus* Pennant (Crustacé Décapode). Effects de l'ablation des pédoncules oculaires. *Arch. Zool. Exp. Gén.* **117:** 117-138.

KEEBLE, F. W., AND F. W. GAMBLE. 1904. The colour-physiology of higher Crustacea. *Phil. Trans. R. Soc. Lond., Ser. B. Biol. Sci.* **196:** 295-388.

LAMBERT, D. T., AND M. FINGERMAN. 1978. Colchicine and cytochalasin B: further characterization of their actions on crustacean chromatophores using the ionophore A23187 and thiol reagents. *Biol. Bull.* **155:** 563-575.

LAMBERT, D. T., AND M. FINGERMAN. 1979. Evidence implicating calcium as the second messenger for red pigment-concentrating hormone in the prawn *Palaemonetes pugio. Physiol. Zool.* **52:** 497-508.

MCNAMARA, J. C. 1976. *The structural basis of pigment migration in the chromatophores of the shrimp Palaemon affinis.* M. Sc. Thesis, University of Canterbury, 2 Vols. 71 pp.

MCNAMARA, J. C. 1979. Ultrastructure of the chromatophores of *Palaemon affinis* Heilprin (Crustacea, Decapoda). Modifications in the shape of hindgut chromatophores associated with pigment movements. *J. Exp. Mar. Biol. Ecol.* **40:** 193-199.

MCNAMARA, J. C. 1980. Ultrastructure of the chromatophores of *Palaemon affinis* Heilprin (Crustacea, Decapoda). The structural basis of pigment migration. *J. Exp. Mar. Biol. Ecol.* **46:** 219-229.

McNamara, J. C. 1981a. Ultrastucture of the chromatophores of *Palaemon affinis* Heilprin (Crustacea, Decapoda). The hindgut chromatophores. *Bol. Fisiol. Animal, Univ. São Paulo* **5:** 7–17.

McNamara, J. C. 1981b. Ultra-estrutura comparativa de cromatóforos em crustáceos decápodes. Ph.D. Thesis, University of São Paulo. 52 pp., 104 figs.

Nicol, J. A. 1967. *The biology of marine animals.* Sir Isaac Pitman & Sons Ltd, London. 699 pp.

Parker, G. H. 1948. *Animal colour changes and their neurohumours.* Cambridge University Press, Cambridge. 377 pp.

Pouchet, G. 1876. Des changements de coloration sous l'influence des nerfs. *J. Anat. Physiol.* **12:** 1–90.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17:** 208–212.

Robison, W. G., Jr, and J. S. Charlton. 1973. Microtubules, microfilaments, and pigment movement in the chromatophores of *Palaemonetes vulgaris* (Crustacea). *J. Exp. Zool.* **186:** 279–304.

Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26:** 31–43.

Reference: *Biol. Bull.* **161**:281–290. (October, 1981)

DICLIDOPHORA NEZUMIAE SP. N. (MONOGENEA: DICLIDOPHORIDAE) AND ITS ECOLOGICAL RELATIONSHIPS WITH THE MACROURID FISH *NEZUMIA BAIRDII* (GOODE AND BEAN, 1877)*

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ABSTRACT

Diclidophora nezumiae sp. n. is described from the gills of the rat-tail fish *Nezumia bairdii* (Goode and Bean, 1877) taken from the environs of Hudson Submarine Canyon in the northwest Atlantic. The host-parasite relationships were studied in the host population. The new species is most similar to small species of *Diclidophora* having short bodies that taper to maximum width at the level of the first pair of clamps. It may be differentiated from other species by the following: clamps wider than long, noticeably decreasing in size posteriorly; lamellate extension of sclerite *b* does not fuse with sclerite c^1; unsclerotized diaphragm; a relatively small clamp sucker; 10–13 cirrus hooks; 18–30 intercecal, postovarian testes; unlobed seminal receptacle; filamented eggs; and body dimensions. Of 378 *N. bairdii* specimens examined, 106 (28%) were infected with 1–21 *D. nezumiae* per host. The parasite occurred most frequently on filaments of the first gill arch. Infected fish ranged from 61–428 mm in total length. They were collected at depths of 300–1900 m. Both incidence and intensity of infection were greater for hosts collected between 700–1000 m. Depth of capture of the host was more strongly correlated with fish abundance than with fish size.

INTRODUCTION

Campbell *et al.* (1980) found few species of monogenetic trematodes among the metazoan parasites from 1712 (52 spp.) deep-living benthic fishes taken in the environs of Hudson Submarine Canyon in the northwest Atlantic. Despite examinations of larger (older) fish from depths as great as 5000 m, they found trematodes particularly rare at bottom depths of 2500 m or more, where host populations were low. Most hosts were gregarious and more numerous in shallower habitats. Such relationships were particularly true of *Nezumia bairdii* (Goode and Bean, 1877), infected with a new species of *Diclidophora, D. nezumiae,* described herein. This paper provides additional observations on *D. nezumiae's* occurrence among fish of varying size (age), and on its distribution on the gills of its host and in the host's deep-sea habitat.

In the Hudson Canyon area, *Nezumia bairdii* is the most abundant macrourid

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Abbreviations: TL, total length; A. F. A., alcohol-formalin-acetic acid.

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in the shallower and mid-depth regions. But its abundance decreases markedly at depths in excess of 1000 m (Marshall and Iwamoto, 1973; Haedrich and Polloni, 1974; Haedrich, *et al.*, 1980). The species is most common along the Atlantic coast of North America at depths of 90–2285 m. In northern regions it enters cold waters to depths as shallow as 90–183 m, but in lower latitudes it is more common at depths of 548–731 m (Leim and Scott, 1966; Marshall and Iwamoto, 1973).

Materials and Methods

We examined 378 specimens of *N. bairdii* ranging from 5–43.5 cm TL (total length) for parasites and found 347 specimens of *D. nezumiae* sp. n. on 106 infected fish. Hosts examined were as large as any reported (40 cm TL; Marshall and Iwamoto, 1973). Except for the absence of juveniles smaller than 34 mm TL, they represented the complete size range known for the fish species. Fish were collected from most of the species' known depth range (260–1965 m sample range) as part of faunal zonation studies (Rowe and Haedrich, 1979; Haedrich *et al.*, 1980) in Hudson Submarine Canyon (39°13'N; 71°55'W) and adjacent regions of the continental slope and shelf areas. Collecting gear used included semi-balloon, shrimp, or beam trawls.

Helminths were obtained from freshly caught fish, those placed on ice, or from fish preserved in 10% neutral-buffered formalin and transferred to 70% ethanol. Fixing solutions were A.F.A. (alcohol-formalin-acetic acid) or 10% neutral buffered formalin. Whole mounts were stained in Mayer's paracarmine, dehydrated, and mounted in Canada balsam. Type specimens were deposited in the U. S. National Parasite Collection, Beltsville, Maryland, and in the British Museum (Natural History).

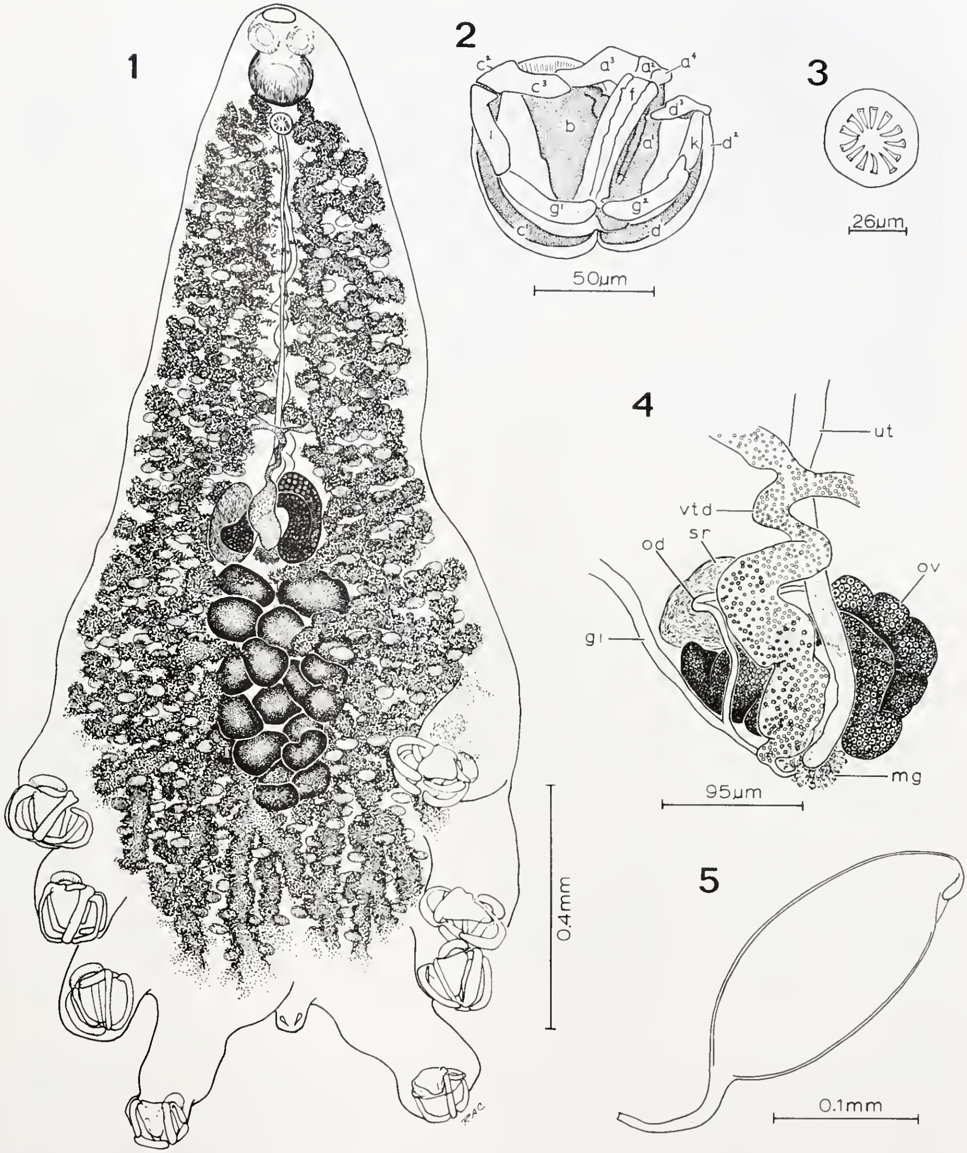
A statistical computer package was used to analyze the data (Nie *et al.*, 1975). Intensity data followed a negative binomial distribution and were log transformed to approximate normality. Transformed data were analyzed by linear least-squares regression analysis, one-way analysis of variance, chi-square analysis and the Student-Newman-Keuls multiple range test. Descriptive measurements are expressed as length by width with means given in parentheses. All measurements are in micrometers unless stated otherwise.

Description

Diclidophora nezumiae sp. n. (Figs. 1–5)

Diagnosis: based on 347 specimens, 15 measured. Small worms, broadly triangular in shape, total length 980–2.4mm (1.52 mm) by 420–980 (643) with maximum width at posthaptor. Prohaptor consists of two buccal suckers, 42–61 (50) by 34–53 (41). Opisthohaptor not set off from body proper, bearing four pairs of clamps of unequal size on short peduncles and a terminal lappet. Anterior clamps largest, 76–129 (100) by 106–144 (126); posterior clamps smallest, 72–86 (81) by 80–95 (88); one to three pairs of small hooks sometimes present on terminal lappet. Clamps possess opposable jaws and eight sclerites as described by Llewellyn (1958); lamellate extension b of sclerite a^1 not fused with sclerite c^1, muscular pad over ring formed by sclerites a^2, a^3, b, c^2, c^3; sclerites a^3 and c^3 approach each other medially; d^3 of anterior jaw closely approximating median sclerite a; posterior jaw reinforced by weakly developed riblike thickenings, sclerites g^1 and g^2 rather short and curved to meet sclerites i and k; no lateral flange on sclerite f.

Mouth small, subterminal, ventral. Pharynx 68–117 (102) by 72–91 (85). Esophagus short, intestinal bifurcation just anterior to genital pore. Ceca forming


1
2
c²
c³
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50µm
3
26µm
4
ut
vtd
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od
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mg
95µm
5
0.1mm
0.4mm
RAC

FIGURE 1. *Diclidophora nezumiae* sp. n., holotype. FIGURE 2. Clamp, posterior view. FIGURE 3. Cirrus armature. FIGURE 4. Detail of female reproductive system: gi, genito-intestinal canal; mg, Mehlis' gland; od, oviduct; ov, ovary; sr, seminal receptable; ut, uterus; vtd, vitelline duct. FIGURE 5. Egg, *in utero*.

dorsal and ventral series of medial and lateral diverticula; some diverticula confluent posterior to testes; branches entering posterior extremity but terminating at origins of clamp peduncles.

Testes subspherical or irregular, 18–30 in number, in postovarian median field, entering posthaptor to level of first or second pair of clamps. Vas deferens passing anteriorly in midline to enter muscular cirrus; cirrus 38–48 in diameter, armed with 10–13 (11) recurved hooks.

Ovary N- or U-shaped, median, in posterior one-half of body. Seminal receptacle and genito-intestinal canal on right side of ovary. Mehlis' gland conspicuous, immediately posterior to ovary. Vitelline follicles small and numerous, coextensive with ceca, not confluent dorsal to testes; transverse vitelline ducts joining in midline just anterior to ovary to form vitelline resevoir. Uterus ascending anteriorly in midline, dorsal to vitelline resevoir, terminating at genital pore. Eggs rarely seen, solitary, filamented, elliptical 216–280 by 72–95 exclusive of filaments.

Systematic relations

Diclidophora nezumiae sp. n. is similar to small species of *Diclidophora* in which the posthaptor is not set off from the body, and which have a triangular body that tapers to maximum width at the level of the first pair of clamps. As might be expected, species most similar to *D. nezumiae* are also from macrourid hosts. Those species are *D. macruri* Brinkmann, 1942, *D. coelorhynchi* Robinson, 1961, and *D. paracoelorhynchi* Mamaev, Parukhin and Zubchenko, 1979. *Diclidophora nezumiae* may be differentiated from these and other species of *Diclidophora* by: 1) short, triangular body not subdivided from the posthaptor; 2) ovary located at midbody to the left of the saccate seminal receptacle; 3) 10–13 cirrus hooks; 4) 18–30 testes confined to the postovarian intercecal space and extending posteriorly to the second pair of clamps; 5) filamented eggs; 6) clamps wider than long and decreasing in size posteriorly; and, 7) unsclerotized clamp diaphragm and lamellate extension *b* not fused with c^1 and diaphragm. *Diclidophora macruri,* a species Campbell *et al.* (1980) found on the gills of *Coryphaenoides rupestris* Gunnerus, 1765, differs in having clamps distinctly longer than wide. Morphological differences of *D. coelorhynchi* include its 18 cirrus hooks, and pedunculated clamps in which the diagragm is not quite united with the lateral sclerites or the base of the central sclerite, so that no ring is formed to support the sucker. The general morphology and clamp structure of *D. paracoelorhynchi* are closest to *D. nezumiae. D. paracoelorhynchi's* clamp structure is virtually identical in form to that of *D. nezumiae,* except that *D. paracoelorhynchi* has a much larger and more powerful muscular sucker in each clamp than *D. nezumiae* (compare Fig. 1B, Mamaev *et al.*, 1979, with *D. nezumiae*). Furthermore, specimens of *D. paracoelorhynchi* are up to twice as large as *D. nezumiae,* have a lobed seminal receptacle and 40–60 testes.

Of the species of *Diclidophora* from non-macrourid teleosts, *D. phycidis* (Parona and Perugia, 1889), *D. luscae* (Beneden and Hesse, 1863), and *D. esmarkii* (Scott, 1901) resemble *D. nezumiae.* They may be differentiated from the new species in the following ways: 1) *D. luscae* and *D. esmarkii* (morphologically indistinguishable according to Llewellyn and Tully, 1969) have tubercles on the surface of the clamp jaw, para- and postovarian testes and 8–10 cirrus hooks; and 2) *D. phycidis* has many para- and postovarian testes that intrude deeply into the posthaptor (see Llewellyn and Tully, 1969), a well developed sclerotized disc in the diaphragm, an anterior seminal receptacle, unfilamented eggs, and 8–10 cirrus hooks.

RESULTS

Most reports of Monogenea from macrourid fishes have been taxonomic in nature, and, because of the small number of hosts examined, have provided little information on the ecological interactions between host and parasite populations (see Brinkmann, 1942; Robinson, 1961; Radulescu, 1969; Orias and Noble, 1971; Noble, 1973; Armstrong, 1974; Mamaev *et al.* 1979; Lambert and Euzet, 1980). Earlier reports on Monogenea from macrourids in the genus *Nezumia* listed only unidentified parasites (Radulescu, 1969; Orias and Noble, 1971; Nobel, 1973). Orias and Nobel (1971) found 12 specimens of an unidentified species of Mazocraeoidinae from four *N. bairdii* adults collected off Greenland. Recently, Lambert and Euzet (1980) described a new genus and species of Monogenea (*Polycliphora nezumiae*) parasitic on *N. aequalis* collected near Senegal in the eastern Atlantic.

Regression analysis of fish TL versus depth of capture for the 378 *N. bairdii* specimens examined in this study indicated that fish size increased with increasing depth ($Y = 99.52 + 07.96X$; $r^2 = 0.51$). Because of this "bigger-deeper" relationship, parasite data were analyzed for depth-related and host-size-related differences in incidence and intensity of infection. Table I shows that 28% (106) of the fish were infected with a single species of Monogenea, *D. nezumiae.* A total of 347 *D. nezumiae* specimens were found from fish captured between 300–1900 m, and ranging from 61–428 mm TL.

Incidence

Table I shows incidence data for *D. nezumiae* on fish arranged by 100 m depth intervals. Pooled data (Table II) shows that fish captured between 700–1000 m were more often infected (62%) by *D. nezumiae* than fish taken at other depths.

Comparison of parasite incidence versus host size in each depth zone (Table III) showed that fishes from 700–1000 m had infections more often, regardless of fish size, than those collected from other depths. Larger fishes (>210 mm TL) in every depth zone, except those taken below 1100 m, were more often infected than smaller fish from the same depth zone. Infections were infrequent on all hosts living below 1100 m.

Pooling infection data by fish size regardless of depth of capture (Table IV) indicated that the highest incidence of infection (37%) was in fish ranging from 110–300 mm TL. Immature worms were obtained from seven fish ranging from 83–255 mm TL. Six of these hosts were collected at 500 m and one from 1500 m,

TABLE I

Summary of infection data for Diclidophora nezumiae *on* Nezumia bairdii *collected in the Hudson Canyon area.*

Depth	x̄ Host size (mm)	Examined/ infected	% Infected	x̄ Intensity	No. of worms recovered	No. of multiple infections
300– 400	114	35/10	29	2.8	28	7
500– 600	160	125/44	35	3.0	132	25
700– 800	246	21/12	57	5.2	62	8
900–1000	238	32/21	66	3.3	70	11
1100–1200	249	3/ 2	67	1.0	2	0
1300–1400	305	139/ 9	6	3.6	32	6
1500–1600	304	6/ 1	17	4.0	4	1
1700–1800	190	7/ 2	29	1.5	3	1
1900	324	10/ 5	50	2.8	14	3
Totals		378/106	28	3.3	347	62

TABLE II

Summary of incidence data for Didlidophora nezumiae *on* Nezumia bairdii. *Data from Table I has been pooled for three depth zones.*

Depth	x̄ Host size (mm)	Examined/infected	% Infected*
300– 600	150	160/54	34
700–1000	184	53/33	62
1100–1900	300	165/19	12
Total		378/106	28

* Differences in frequency of occurrence were significant ($\chi^2 = 51.71$; $p < 0.01$) for each depth zone.

indicating that *D. nezumiae* is recruited throughout the life of *N. bairdii* in all depths of its habitat.

Intensity

In general, each host harbored an average of 3.3 worms with a range of 1–21 worms among all hosts (Table I). Parasite-free fish and those with only a single monogenean were most common at every depth. Multi-worm infections of *D. nezumiae* occurred on 62 of 106 (58%) hosts, the bulk of them collected from 500–1000 m.

Only a poor correlation ($r = -0.03$) was found between depth of capture of the host and intensity of infection. Also, no significant differences were found when the pooled parasite data were analyzed (one-way ANOVA) from the three depth intervals (Table III). Fishes captured between 700–1000 m had the heaviest average parasite burdens, compared to hosts from other depths.

Significant size-related differences ($p < 0.05$) in intensities were found when

TABLE III

Infection data for Diclidophora nezumiae *on* Nezumia bairdii. *Data for hosts is divided into three size groups from three depth zones.*

Fish size (mm)	# Examined/infected	% Infected	Mean intensity
DEPTH 300–600 m			
50–200	126/33	26	1.85
210–300	28/18	64	4.67
310–375	6/ 3	50	5.33
DEPTH 700–100 m			
70–200	17/ 7	41	1.29
210–300	24/16	67	4.31
310–355	12/10	83	6.30
DEPTH 1100–1900 m			
34–200	8/ 2	25	1.00
210–300	79/12	15	3.33
310–435	78/ 4	5	3.00

TABLE IV

Incidence and intensity of infection of Diclidophora nezumiae *on 378* N. bairdii *specimens grouped into four size classes independent of depth of capture. Fish lengths are total lengths expressed in millimeters.*

	Size class (mm)				
	34–100	110–200	210–300	310–435	Total
Number examined	62	90	132	94	378
Number infected	9	33	48	16	106
Percent infected	14	37*	36*	17	28
Mean intensity**	144a	1.76a	4.04b	5.12c	3.3
Range	1–3	1–5	1–21	1–16	1–21

* Differences in frequency of occurrence of infections between intermediate-sized fishes and other size-groups were significant ($\chi^2 = 13.81$; $p < 0.01$).

** Values succeeded by the same letter are not significantly different ($p < 0.05$) (Student-Newman-Keuls multiple range test).

four different size groups of hosts were compared regardless of capture depth (one-way ANOVA; Table IV). Intensity of infection increased with host size.

Size-related differences in intensities of infection were also evident when data were compared within a given depth zone (Table III). In every depth zone the two groups of larger fish consistently had greater numbers of *D. nezumiae* than smaller fishes collected from the same depths. Fishes ranging from 34–200 mm TL were infected by similar numbers of worms regardless of capture depth. Intermediate-sized fish (210–300 mm TL), collected from 300–600 m and 700–1000 m, were infected with similar numbers of worms. The latter parasite densities were considerably greater than those on fishes of similar size taken below 1100 m. *N. bairdii* specimens of more than 310 mm TL taken from 700–1000 m had the highest intensity of infection ($\bar{x} = 6.3$) of any size group examined. Large fish taken from 300–600 m had similar parasite densities, but those taken below 1100 m had only one-half the number of Monogenea per fish compared to those from 700–1000 m.

Microhabitat

Data on microhabitat of *D. nezumiae* collected from 28 hosts are presented in Tables V–VI. Since chi-square analysis indicated no significant differences ($p > 0.30$) between number of infections of arches on right or left sides, data from

TABLE V

Distribution of Diclidophora nezumiae *on the gill arches of* Nezumia bairdii *(Percent occurrence in parentheses; number of hosts = 28).*

	Gill arch					
	I	II	III	IV	Unknown	Total
No. worms—right side	17(22)	8(10)	1(1)	10(13)	6(8)	42(53)
No. worms—left side	22(28)	4(5)	2(2)	6(8)	3(4)	37(47)
Total	29(49)	12(15)	3(4)	16(20)	9(11)	79(100)

Table VI

Distribution of mature and immature Diclidophora nezumiae *specimens on particular gill arches and hemibranchs of* Nezumia bairdii *(Percent occurrence in parentheses).*

	No. of worms	Gill arch				No. of worms	Hemibranch		Gill Arch Position	Incidence	
		I	II	III	IV		Outer	Inner		Mature	Immature
Mature	57	33(47)	7(10)	3(4)	14(20)	54	42(63)	12(18)	Dorsal	16	3
Immature	13	6(9)	5(7)	0(0)	2(3)	13	5(8)	8(12)	Middle	9	2
Total	70	39(56)	12(17)	3(4)	16(23)	67	47(70)	20(30)	Ventral	7	2

both sides were pooled. The dorsal one-third of gill arch I was most often infected and was the site selected by most of the mature and immature worms (50% of total). Gill arches II and IV were each infected 11 times with similar numbers of worms, and gill arch III was the least often and least heavily infected site ($\chi^2 = 9.43$; $p < 0.05$). Mature worms were more often observed attached to the outer (anterior) hemibranchs than the inner (posterior) ones, but immature worms were distributed evenly between the hemibranchs. All worms attached to a single primary lamella in a manner similar to that described for *D. luscae* (Llewellyn, 1956).

Discussion

Parasite populations, particularly those which do not use intermediate hosts in their life cycles, are especially susceptible to variations in their hosts' population densities. Examination of the distribution of infections by *D. nezumiae* on *N. bairdii* revealed that interactions of parasite and host populations varied with capture depth and host size.

Nezumia bairdii has a "bigger-deeper" distribution in the Hudson Canyon area (Polloni *et al.*, 1979; Haedrich *et al.*, 1980). Within this area, these fish occurred in three distinct depth zones. In the shallowest zone (300–600 m), the *N. bairdii* population was predominantly smaller fish ranging from 50–160 mm TL ($\bar{X} = 150$ mm TL). The second zone, 700–1000 m, was inhabited mainly by intermediate-sized fishes, 170–300 mm TL ($\bar{X} = 184$ mm TL). The third depth zone, 1100–1900 m, was inhabited by large fish, usually >250 mm TL ($\bar{X} = 300$ mm TL). Haedrich *et al.* (1980) and Marshall and Iwamoto (1973) noted that *N. bairdii* decreases in abundance below 1000 m. As noted earlier, within the Hudson Canyon area the center of abundance for *N. bairdii* lies between 500–1000 m.

In view of the dispersion of the different size groups of *N. bairdii,* it was not surprising to find different incidences of parasitism by *D. nezumiae* on hosts collected from different depths. Analysis of the infection data (both incidence and intensity) showed that the highest levels of parasitism by *D. nezumiae* occurred on fish collected from 700–1000 m, the depth zone corresponding to the center of abundance for *N. bairdii* in this region (Haedrich *et al.*, 1980). Each of the three size groups within this depth zone was infected more often and usually harbored greater numbers of worms per fish than their counterparts captured at other depths. The high levels of infection in fishes collected between 700–1000 m indicates that this depth zone is also the center of abundance for *D. nezumiae.* Hence, the success and abundance of *D. nezumiae* depends directly upon the host population density.

Host-size was secondary in importance to depth-related differences in host population densitites in influencing infections of *D. nezumiae.* Only fishes smaller than

60 mm TL were completely free of monogeneans. All other size groups harbored *D. nezumiae,* but in numbers varying with host size. The smallest *N. bairdii* specimens (34–200 mm TL) had the lowest infection rate (14%) and, with one exception, harbored the fewest worms per fish ($\bar{x} = 1.6$ per host) of any size group examined. Low intensities of infection may be expected in these smaller fish because they were collected mostly in depths less than 700 m (shallower than the center of abundance of *N. bairdii*) and because the smallest of these fishes probably represented recent recruits settling from overlying waters. New recruits evidently had not had sufficient time to develop infections of *D. nezumiae.*

Intermediate-sized fish (210–300 mm TL), exception in one depth zone, had the highest infection levels of all those we examined. The exception was in the 700–1000 m zone, where fish >310 mm TL had higher frequencies and intensities of infection. The high levels of infection in the intermediate-sized fishes was expected because a major proportion of these fishes were collected between 700–1000 m, the center of abundance for *D. nezumiae* (Table III).

The largest fish (310 mm TL) were taken mostly below 1000 m. Only 18 of 96 individuals from this size group were collected from depths less than 1100 m. Both incidence of infection and intensity per infection were lower in this size group than in the intermediate-sized fishes. In the deepest area, where 81% of the largest fishes were collected, only 4 of 78 (5%) were infected.

Diclidophora nezumiae had a preferred site of attachment: both adults and immature worms preferred gill arch I. Mature worms preferred the dorsal one-third of the outer hemibranchs, but immature worms were almost evenly distributed between the hemibranchs. Gill arches II and IV were also occupied by mature and immature monogeneans, but to a lesser degree. Where attachment site information was available for other species of *Diclidophora,* only three of six species (*D. denticulata* Rees and Llewellyn, 1941; *D. macallumi* Price, 1943 and D. merlangi (Kuhn, 1832)) were found most often on gill arch I.

Monogeneans' attachment sites are related to a variety of physical and physiological factors. Among these are the host's ventilating mechanism and immune responses, the parasite's mobility, and intraspecific competition among monogeneans (Suydam, 1971). It is not known which of these factors influence the distribution of diclidophorids on their macrourid hosts, but intraspecific competition seems unlikely, as macrourid infections typically involve only a few specimens of Monogenea. In fact, their most likely metazoan competitor upon the gills of macrourids would be parasitic copepods.

This study shows that factors which influence the success and abundance of infection of monogeneans in shallow-water fishes, such as host population density, distribution, and size, also influence the degree of infection attained by monogenetic trematodes in slope-dwelling fishes such as *N. bairdii.*

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LITERATURE CITED

ARMSTRONG, H. W. 1974. *A study of the helminth parasites of the family Macrouridae from the Gulf of Mexico and Caribbean Sea: Their systematics, ecology, and zoogeographical implications.* Doctoral dissertation. Texas A & M University. 329 pp.

BRINKMANN, A., JR. 1942. On some new and little known *Dactylocotyle* species, with a discussion on the relations between the genus *Dactylocotyle* and the "Family" Diclidophoridae. *Goteborgs Mus. Zool. Avdelnig. (92). Goteborgs K. Vetensk-O. Handl. 6 (Ser. B, Bd 1)* **13:** 1–32.

BRINKMANN, A., JR. 1952. Fish trematodes from Norwegian waters. I. The history of fish trematode investigations in Norway and the Norwegian species of the order Monogenea. *Arbok Bergen (Naturvit. Rekke)* **1:** 1–134.

CAMPBELL, R. A., R. L. HAEDRICH, AND T. A. MUNROE. 1980. Parasitism and ecological relationships among deep-sea benthic fishes. *Mar. Biol.* **57:** 301–313.

HAEDRICH, R. L., AND P. T. POLLONI. 1974. Rarely seen fishes captured in Hudson Submarine Canyon. *J. Fish. Res. Bd. Can.* **31:** 231–234.

HAEDRICH, R. L., G. T. ROWE, AND P. T. POLLONI. 1980. The magabenthic fauna in the deep-sea south of New England, U. S. A. *Mar. Biol.* **57:** 165–179.

LAMBERT, M., AND L. EUZET. 1980. *Polycliphora nezumiae* gen. et sp. nov. (Monogenea: Diclidophoridae) a gill parasite of *Nezumia aequalis* (Macrouridae) from Senegal. *Parasitology* **80,** 413–417.

LEIM, A. H., AND W. B. SCOTT. 1966. Fishes of the Atlantic coast of Canada. *Bull. Fish. Res. Bd. Can.* **155:** 1–485.

LLEWELLYN, J. 1956. The host-specificity, microecology and comparative morphology of some trematode gill parasites. *J. Mar. Biol. Assoc. U. K.* **35:** 113–127.

LLEWELLYN, J. 1958. The adhesive mechanisms of monogenetic trematodes. The attachment of the species of Diclidophoridae to the gills of gadoid fishes. *J. Mar. Biol. Assoc. U. K.* **37:** 67–79.

LLEWELLYN, J., AND C. M. TULLY. 1969. A comparison of speciation in diclidophoridean monogenean gill parasites and in their fish hosts. *J. Fish. Res. Bd. Can.* **26:** 1063–1074.

MAMAEV, Y. L., A. M. PARUKHIN, AND A. V. ZUBCHENKO. 1979. *Diclidophora paracoelorhynchi* and *D. attenuata,* new species of monogeneans from macrourid fish. *Trudy gel'mint. Lab., Moscow* **29:** 97–102 (in Russian).

MARSHALL, N. B., AND T. IWAMOTO. 1973. Family Macrouridae. Pp. 496–662 in *Fishes of the Western North Atlantic.* Number I. Part 6. Mem. Sears Found. Mar. Res., New Haven.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENER, AND D. H. BENT. 1975. *Statistical package for the social sciences.* 2nd Ed. McGraw-Hill Book Co., New York. 675 pp.

NOBLE, E. R. 1973. Parasites and fishes in a deep-sea environment. Pp. 121–195 in F. S. Russell and M. Yonge, Eds., *Advances in marine biology,* Volume 11. Academic Press, London.

ORIAS, J. D., AND E. R. NOBLE. 1971. *Entamoeba nezumia* sp. n. and other parasites from a North Atlantic fish. J. Parasitol. **57:** 945–947.

POLLONI, P. T., R. HAEDRICH, G. ROWE, AND C. H. CLIFFORD. 1979. The size-depth relationship in deep ocean animals. *Int. Revue Ges. Hydrobiol. Hydrogr.* **64:** 39–46.

RADULESCU, I. 1969. Contributii la cunoasterea parazitolor pestilor din Oceanul Atlantic. *Bul. Inst. Cer. Pisc.* **28:** 77–82. (In Italian)

ROBINSON, E. S. 1961. Some monogenetic trematodes from marine fishes of the Pacific. *Trans. Am. Microsc. Soc.* **80:** 235–266.

ROWE, G. T., AND R. L. HAEDRICH. 1979. The biota and biological processes of the continental slope. *Spec. Publs Soc. Econ. Paleont. Miner. Tulsa* **27:** 49–59.

SUYDAM, E. L. 1971. The micro-ecology of three species of monogenetic trematodes of fishes from the Beaufort-Cape Hatteras area. *Proc. Helminth. Soc. Wash.* **38:** 240–246.

Reference: *Biol. Bull.* **161**:291–302. (October, 1981)

LIFE HISTORY CHARACTERISTICS OF *THERMOSPHAEROMA THERMOPHILUM*, THE SOCORRO ISOPOD (CRUSTACEA: PERACARIDA)

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ABSTRACT

The endangered species (Federal endangered species list), *Thermosphaeroma thermophilum*, or "Socorro Isopod," is endemic to a small spring near Socorro, New Mexico, that is thermally stable year-round. Isopods were observed in the field, and monthly samples were collected between March 1978 and February 1979 for laboratory examination. Males were larger than females, and sex ratio in the habitat was consistently biased toward males, particularly when sexually receptive females were abundant. Reproduction occurred primarily, although not exclusively, in spring and fall. Food seemed scarce, and intense predation by the omnivorous isopods appeared to exclude most invertebrate species, including predaceous aquatic insects, from the habitat. Fish do not inhabit the spring and avian predation is minimal or non-existent, perhaps permitting isopods to reach high densities and decreasing risks associated with male mate-searching behavior. Field and laboratory data indicated that isopods live less than 1 year. Males grow and reach sexual maturity faster than females. No evidence of hermaphroditism or sex-change was observed. Females were iteroparous and brood size increased with a female's age, but females were small and varied little in size, suggesting selection for an optimal female size.

INTRODUCTION

The "Socorro Isopod," *Thermosphaeroma thermophilum*, has received considerable popular attention as a result of its recently acquired status as an endangered species (Federal Register, 1977). The isopod has only briefly been mentioned in scientific literature, however, and all previous investigations have been primarily taxonomic (Richardson, 1897, 1900, 1904, 1905; Van Name, 1936; Rioja, 1950; Cole and Bane, 1978).

Thermosphaeroma thermophilum is one of four congeneric species of freshwater sphaeromatid isopods inhabiting thermal springs in the southwestern United States and in Mexico (Cole and Bane, 1978). Life history data on North American freshwater isopods are limited to a handful of papers concerning asellote species (Allee, 1914, 1927; Markus, 1930; Hatchett, 1947; Ellis, 1961), thus, the biology of the entire genus, *Thermosphaeroma*, is virtually unknown.

Thermosphaeroma thermophilum is endemic to a single effluent near Socorro, New Mexico, in which it is one of the few permanent macroscopic animal species. This unusual ecologic situation imposes selective pressures rarely encountered by most organisms. In addition, the habitat is small, isolated and stable, making it

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possible to collect life history information easily and accurately in the field and in the laboratory.

MATERIALS AND METHODS

The habitat of *T. thermophilum* consists of two concrete pools and the plumbing system of an abandoned bathouse called Sedillo (Evergreen) Spring (see Shuster 1979 for details). These impoundments were constructed in the early 1900's when the spring was used by local residents as a recreation site. Before that time, the spring issued from an underground gallery and flowed eastward toward what is now the city of Socorro. The range of the isopod then is unknown, but is not likely to have extended much beyond a point where water temperature remained stable year-round (see discussion). Much of the isopod population is now confined to the larger of the two pools (2.69 × 0.94 m), whose flat bottom is covered with 1–4 cm of finely divided substrate. Isopods burrow into this material during the day and emerge at dusk, frequently reaching high densities (210 individuals/100 cm^2). Vegetation throughout the habitat is limited to an almost indistinguishably thin film of blue-green algae that covers most surfaces. Cottonwood, juniper, and mesquite stand near the spring and contribute detritis. Fish do not inhabit Sedillo Spring, and water in the spring appears deep enough (15–25 cm) to minimize or prevent predation by local birds such as sparrows or quail. Birds were never seen feeding on isopods in over 300 hours of observation (Shuster, 1979).

Field studies

With authorization by the New Mexico Department of Game and Fish, isopods were collected from the larger pool in the 3rd week of each month (except September and November) beginning in March 1978 and ending in February 1979. Water temperatures from three locations within the habitat (Fig. 1a) were taken on each collection date, as was the water temperature of a swimming pool continuous with, though located 20 m southeast of, the habitat. Isopod samples were taken in three random sweeps along the pool bottom with a fine-mesh, 100 × 150 mm square net, immedately placed in an insulated jug containing spring water, and transported to the laboratory, where they were sexed. Females were identified by the presence of a ventral brood pouch (marsupium) and were classified by reproduction condition as (1) gravid (females carrying young in the marsupium), (2) undifferentiated (females possessing marsupial lamellae but with little ovary or hepatopancreas development), or (3) mature (females with large yellow ovaries and a dark distinct hepatopancreas). Both ovaries and hepatopancreas were clearly visible through the ventral cuticle. Males were often larger than females, and possessed a bifurcate penis located medially on the seventh peraeonal segment. Testes were frequently visible through the cuticle in segments 5–7. Individuals less than 4.0 mm in length usually lacked sexual characters and were classified as immature. All mature individuals were measured (length × width) with calipers to the nearest 0.5 mm. This procedure gives a more accurate estimation of individual body size than merely measuring length or width (Thornhill, 1977, 1978, 1979), and accounts for slight differences in body size among individuals. While it may be argued that the use of area compounds the variance of the two measurements, the sizes of males and females were compared using a non-parametric Mann-Whitney U test, which does not consider assumptions about parameters such as variance (Zar, 1974).

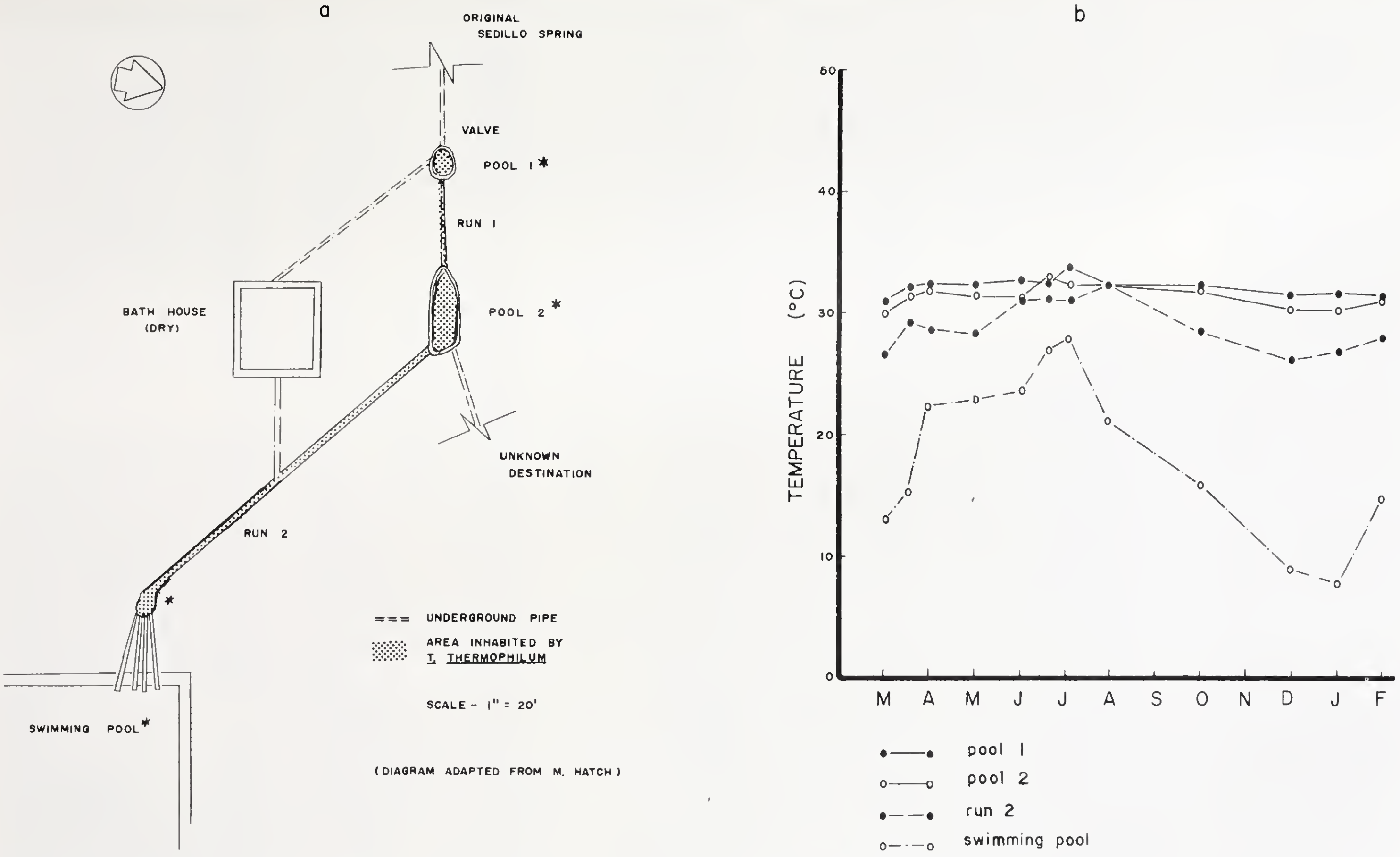


FIGURE 1. (a) The habitat of *T. thermophilum.* (*) indicate locations where water temperature was recorded. (b) Temperatures recorded at Sedillo Spring from March 1978 to February 1979.

The numbers, percentages, and size frequencies of males and females in each reproductive condition (gravid, undifferentiated, mature) were tabulated for each month, and sex ratios were calculated. The number of immatures was also recorded. Several individuals were then dissected and their gut contents microscopically examined, but most individuals were either placed in 35 l holding tanks or returned to the habitat. The feeding behavior of isopods was observed in the field.

Laboratory studies

Individual growth rates were investigated in the laboratory. Twenty-six gravid females were placed in separate chambers constructed of 75 × 100 mm square fish nets and suspended in 35 l aquaria. Water temperature was maintained at 31°C and several flakes of algae fish food (Tetra-min) were added to each chamber every 3–5 days. Algae usually began to grow on the nets within a week or two, and females fed on this growth. Thereafter, fish food was provided only if algae was sparce or absent. Broods were separated from females upon release and counted. Juveniles were measured (length × width) to the nearest 0.1 mm using an ocular micrometer and placed in 75 × 100 mm fish nets with siblings. Individuals were measured each week for 15 weeks, and the appearance of sexual characters was recorded as individuals matured. A logarithmic transformation was used to normalize body size data and growth rates for males and females were fitted to lines using multiple y-value linear regression. The slopes of these lines were compared with a *t* test.

After releasing a brood, each female was returned to her chamber with one mature male. Females were examined weekly for 20 weeks or until they died. Males were removed from chambers when females became gravid, and were returned after each brood was released. Broods were measured and counted as previously described.

RESULTS

Field studies

Temperatures in the portion of Sedillo Spring inhabited by *T. thermophilum* remained relatively constant throughout the year (27°–34° C, Fig. 1b). Temper-

TABLE I

Population demography of T. thermophilum *collected between March 1978 and February 1979 (except during September and November.)*

	Mar.	April	May	June	July	Aug.	Oct.	Dec.	Jan.	Feb.
N	84	162	68	78	147	148	90	80	121	66
No. males (%)	31(36)	128(79)	42(62)	53(68)	117(80)	110(74)	57(63)	47(59)	49(40)	44(67)
Mean male size (mm^2)	13.87	21.78	19.19	22.00	16.12	23.61	26.72	16.02	17.20	16.12
Variance	26.25	38.71	97.49	82.68	77.52	79.32	58.62	38.70	58.00	49.15
No. females (%)	25(30)	33(20)	17(25)	19(24)	23(15)	20(14)	30(33)	15(19)	26(22)	21(31)
Mean female size (mm^2)	10.77	11.27	12.03	12.62	13.87	13.84	15.36	12.47	10.68	8.53
Variance	1.87	6.57	3.79	3.12	2.85	4.18	2.61	7.10	10.68	8.53
No. gravid (%)*	6(24)	22(66)	3(18)	1(5)	2(9)	0(0)	11(36)	3(20)	3(12)	8(38)
No. undiff. (%)*	11(44)	5(16)	5(29)	8(42)	8(34)	4(20)	8(27)	8(53)	9(34)	4(19)
No. mature (%)*	8(32)	6(18)	9(53)	10(53)	13(57)	16(80)	11(37)	4(27)	14(54)	9(43)
No. immatures (%)	28(33)	1(1)	9(13)	6(8)	7(5)	18(12)	3(4)	18(22)	46(38)	1(2)
Sex ratio (m/f)	1.24	3.87	2.47	2.79	5.08	5.50	1.90	3.13	1.88	2.10

* Percentages of the total number of females in each sample.

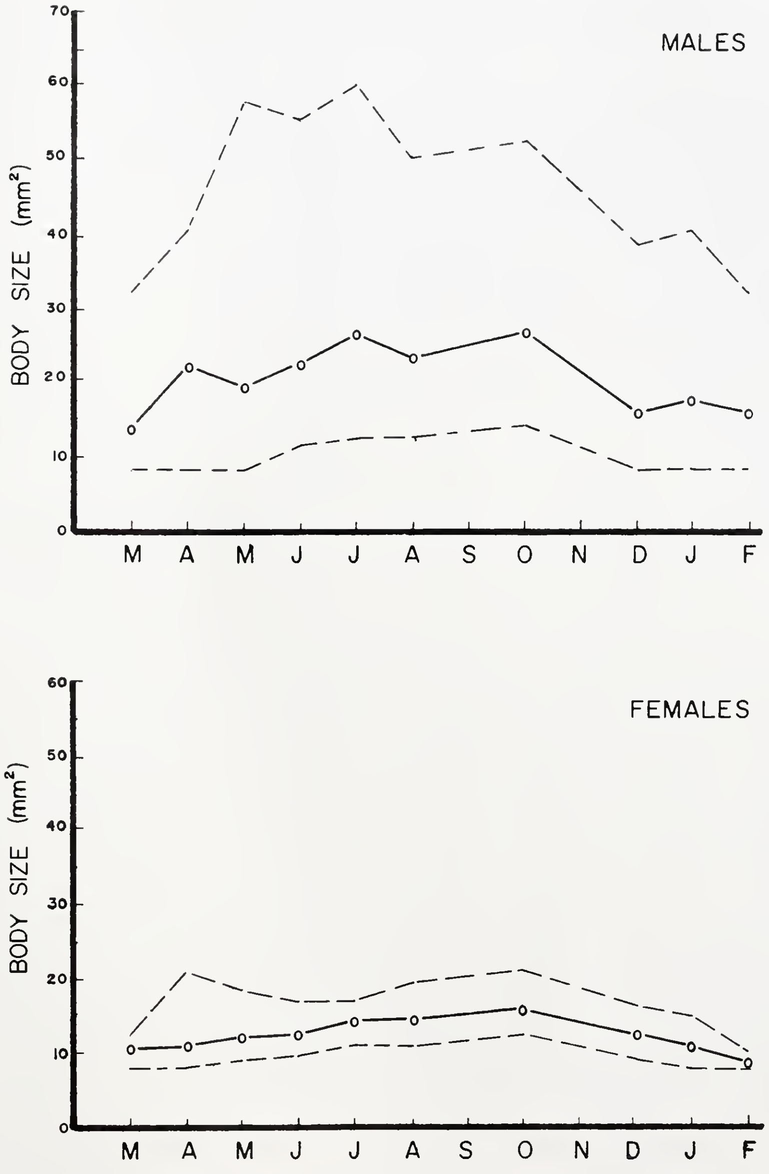


FIGURE 2. Maximum, minimum, and mean sizes of *T. thermophilum* males and females in field-collected samples from March 1978 to February 1979. Upper and lower dashed lines represent maximum and minimum size, respectively. The solid middle line represent mean size.

atures fluctuated considerably, however, in the swimming pool, which was not inhabited by isopods.

Table I summarizes data obtained from monthly samples. Males were consistently more abundant, and significantly larger, than females (males: range = 8.0–60.0 mm^2, median = 19.5 mm^2, N = 678; females: range = 8.0–21.0 mm^2, median = 11.9 mm^2, N = 229, Mann-Whitney U test, $P < 0.005$). Mean body size for both sexes increased from March to October, then decreased rapidly until February.

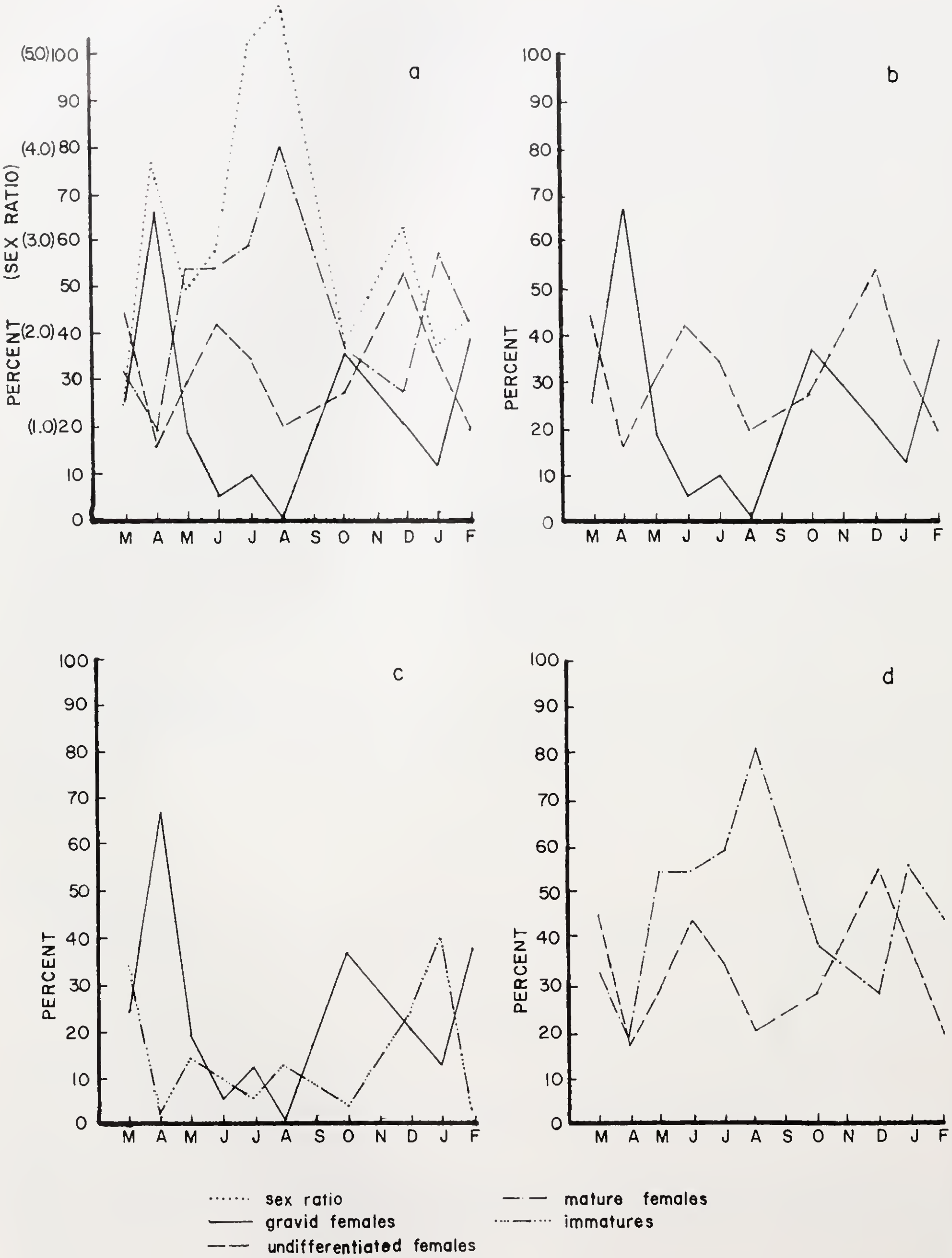


FIGURE 3. (a) Relationship between seasonal variation in sex ratio and seasonal abundance of mature, gravid, and undifferentiated females in field-collected samples from March 1978 to February 1979. Sex ratio is expressed as the number of males/the number of females. Each female type is expressed as a percentage of the total number of females in each sample. (b) Relationship between the seasonal abundances of gravid and undifferentiated females. (c) Relationship between the seasonal abundances of gravid females and immatures. Immatures are expressed as a percentage of the total number of individuals in each sample. (d) Relationship between seasonal abundances of mature and undifferentiated females.

This latter interval also included a conspicuous decrease in the maximum sizes of males and females (Fig. 2). Female size varied only slightly in all samples, but Male size varied considerably.

Sex ratio in the habitat fluctuated dramatically, yet, as mentioned, remained greater than 1:1 (Table I, Fig. 3a). The percentages of females in each reproductive condition also showed much variation. Percentages were used in the figure to represent the relative abundances of different groups, as sample sizes were somewhat unequal. Since percentage data may exaggerate certain relationships, the reader is urged to consult Table I for the actual numbers of individuals in each sample. Gravid females were present in all samples except August, and were particularly abundant in April and in October. As expected, peaks in gravid female abundance were followed by peaks in the abundances of undifferentiated females (Fig. 3b). The abundances of immatures, however, did not follow the abundances of gravid females as closely as expected (Fig. 3c). Mature females were particularly abundant between May and October, seemingly in excess of that expected, given the percentages of undifferentiated females prior to and during this interval (Fig. 3d).

The gut contents of field-caught isopods consisted of finely divided green material with fragments of what appeared to be arthropod cuticle. Two types of feeding behavior were observed in the field: (1) isopods dispersed across the walls and floor of the pool in late afternoon, apparently grazing on blue-green algae, or on bits of grass and other organic debris that had fallen into the water; (2) isopods inadvertently injured in sampling were consumed in "feeding frenzies" by conspecifics. Since injuries occurred on several occasions, I was able to observe such interactions fairly closely. Injured individuals were located within 1–2 min by other isopods, who immediately began feeding on the still-struggling casualty. As more isopods arrived, presumably cueing on blood diffusing into the water, a cluster of 10–30 isopods formed, all vigorously competing for access to the carcass. Individuals on the outside of the cluster would grasp the anterior end of established individuals and peel them backward and off the pile, then rapidly assume the position of the displaced individual. Larger isopods seemed most successful at this manuver. All carcasses were stripped clean, and the feeders dispersed within 1 h.

I also observed two apparently uninjured isopods and two libellulid dragonfly nymphs attacked and consumed in aggregations similar to that described above. Only one intact nymph was ever obtained from sweep samples, although pieces of arthropod cuticle were often found. Other live invertebrates collected in samples were one dytiscid beetle, one belostomatid bug, and numerous oligochaete worms. The five arthropods collected or observed, other than isopods, were less than 5.5 mm in length.

Laboratory studies

A survivorship curve for lab-reared individuals is shown in Figure 4. Nearly 95% died within 15 weeks. Individuals reached sexual maturity between 4 and 11 weeks, and males seemed to mature more rapidly than females (Fig. 5). Males also grew more rapidly than females (t test, $P \ll 0.001$, Fig. 6). No evidence of hermaphroditism or sex-change was observed in this species.

Females are iteroparous and all gravid females (26 original females plus 4 producing more than one brood) released broods between 18 and 51 days after isolation ($\bar{X} \pm SD = 27.30 \pm 7.19$ days, $N = 30$). Of the 26 gravid females first isolated, four died after releasing young. The ovaries of all remaining females (22) became secondarily mature between 18 and 50 days ($\bar{X} \pm SD = 35.0 \pm 12.71$

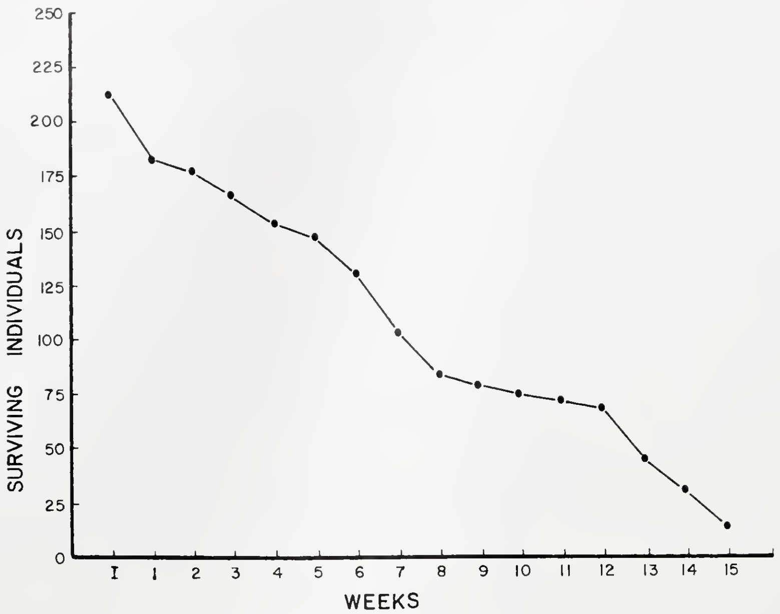
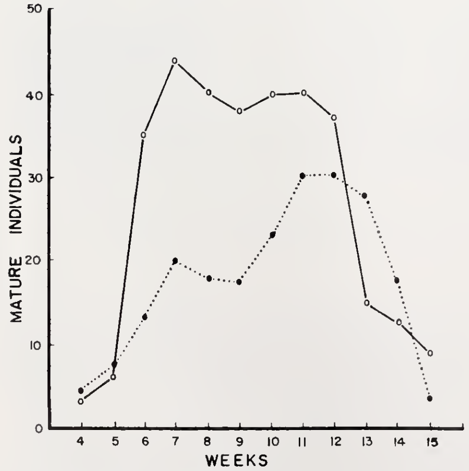


FIGURE 4. Survivorship curve for lab-reared *T. thermophilum.*

days). A heater malfunction killed 5 females, leaving 17, and 4 of these 17 were removed for use in another experiment (Shuster, unpublished). Five of the remaining 13 females became gravid, and 3 of these 5 released a second brood. In all cases, the second brood was larger than the first (Table II).

The ova of females not becoming gravid were apparently resorbed, as were the broods of the two gravid females which did not release offspring. The latter two



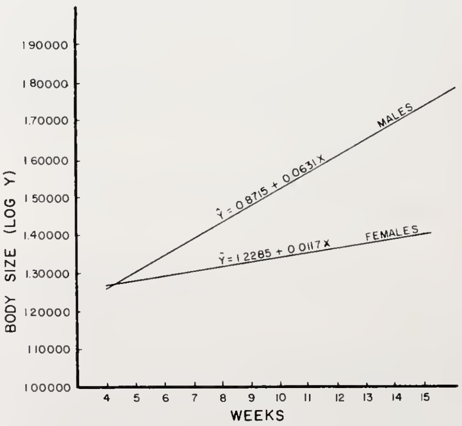


FIGURE 5. Maturation rates for lab-reared *T. thermophilum* males and females. Solid line represents males; dotted line represents females.

FIGURE 6. Growth rates for lab-reared *T. thermophilum* males and females. The slopes of these lines are significantly different (*t* test, $P \ll 0.001$).

TABLE II

Body sizes (in mm²) and brood sizes of females that produced more than one brood in the laboratory.*

Female	Initial size	1st brood (# ind.)	Size after next molt	2nd brood (# ind.)	Size after next molt	3rd brood (# ind.)
C1	12.5	6	16.5	15	—	—
D6	12.5	11	—	—	—	—
A3	12.5	10	—	—	—	—
A4	8.0	6	11.25	9	—	—
B2	11.25	9	15.0	14	19.25	20

females died before their ovaries matured again. The ovaries of two of the three females which released a second brood matured, and one of the two became gravid a third time and released a third brood. This brood was larger than the female's two previous broods (Table II).

DISCUSSION

Most of Sedillo Spring is thermally stable year-round. Temperature fluctuations in most areas inhabited by *T. thermophilum* were 30°–33° C, and water temperature never dipped below 27°C, even at the lower end of the habitat. Isopods do not inhabit a nearby swimming pool where water temperature was considerably lower than in the rest of the spring and varied with ambient air temperature. This suggests that *T. thermophilum* (and probably other *Thermosphaeroma* species) is adapted to thermally stable conditions, and that the range of this species was restricted long before the habitat was modified by man.

Sexual dimorphism in this species is pronounced. I demonstrated earlier (Shuster, 1981) that male-male competition in *T. thermophilum* is intense, and that large males are more successful at obtaining high-quality females (relative quality defined in terms of fecundity and proximity to a sexual molt) as mates than small males. Hence, large male size in this species may be considered a sexually selected attribute. Hansen (1906) states that sexual size dimorphisms are widespread among the Sphaeromatidae. Although behavioral ecology and sexual selection are not often the focus of most isopod research (but see Manning, 1975; Magniez, 1978; and Ridley and Thompson, 1979), I suggest that sphaeromatid sexual dimorphisms are largely, if not exclusively sexually selected (see Veuille and Roualt, 1980), and the family as a whole may provide numerous tests of present evolutionary and sexual selection theory.

Males were more abundant in the habitat than females, and were frequently observed roaming across the pool bottom. Trivers (1972) predicted that males may enhance their reproductive success by searching for mates, even when the costs of searching are high (see Thornhill, 1979; Gwynne and O'Neill, 1980). If such male behavior has been favored in this species, as is reasonable given a high degree of intrasexual competitition and relatively low predation risks (see below), sweep samples are likely to have included a disproportionately large number of males.

The tremendous fluctuations in the abundances of females in various reproductive conditions (Figs. 3b–d) are puzzling and remain largely unexplained. One relationship, however, is clear: gravid females were present in all samples except one, and were particularly abundant in spring and in the fall. This suggests that while reproduction is somewhat seasonal, females reproduce year-round. As expected from laboratory data, peaks in the abundances of undifferentiated females followed peaks in gravid female abundance by about one month. Unexpectedly, however, the number of immatures in May was quite low, perhaps indicating a period of unusually high juvenile mortality. Unfortunately, I have no data to defend or refute this possibility.

Mature females occurred considerably more frequently in samples than expected between May and October, and sex ratios were periodically highly skewed (not merely biased) toward males. Since undifferentiated females become mature within 1–2 months, the dramatic August peak in mature female abundance should have been preceeded by a similar peak for undifferentiated females, perhaps in June or July. While an increase in undifferentiated females was observed in June, it was numerically insufficient to account for the later boom of mature females.

Males guard females before copulation in this species, and prefer females with mature ovaries (Shuster, 1981). Since males apparently determine the reproductive condition of females at close range (*i.e.* by grasping and manipulating them), males routinely grasp nearly every object that is about the same size and shape as a female isopod. Objects or individuals other than mature females are soon discarded, and the male continues searching. Parker (1974) suggested that male guarding behavior could be detrimental to females because of lost feeding time and possible injury, and therefore proposed than these costs should be incurred only by females that are ready to copulate. In turn, non-receptive females should leave areas of high male density. If mature (receptive) females in this species seek areas of high male density, they may have geen sampled more frequently than expected, producing the anomalous relationship between undifferentiated and mature females in Fig. 3d. Concurrently, increased mature female abundance may have precipitated intensified male mate-searching behavior and skewed the August sex ratio sharply toward males (5.50:1, Table I).

The frequency of mature females in April and in December was low, and this, combined with emigration of undifferentiated and gravid females from areas of high male density (by burrowing or hiding in cracks) could also have skewed the sex ratio toward males. If this occurred, however, one might expect an overall decrease in the number of females contained in samples from April and December. This is not readily apparent in Table I. More intensive sampling, especially in potential refugia for non-mature females, may shed light on this matter.

Thermosphaeroma thermophilum is omnivorous. Gut contents consisted of vegetable and animal material, and isopods were observed feeding on both substances in the field. The paucity of vegetation in the spring, and especially the voracity with which isopods consumed their conspecifics and other animals, suggest that food is scarce, or at least that individuals able to obtain such resources gain sufficient fitness to justify vigorous contests for food. Brues (1932) reported considerable diversity of invertebrate species in thermal springs with physical characteristics similar to those in Sedillo Spring. That such diversity in this spring was lacking, suggests that colonizing invertebrates, including young predaceous aquatic insects, are rapidly extirpated via predation by isopods. This, combined with a lack of fish and avian predators, may reduce the costs of male mate-searching behavior. Low predation pressure may also permit isopods to reach high densities in the habitat, and limit surplus food.

That mortality among lab-reared individuals was nearly 95% in 15 weeks is by itself inconclusive. Isopods were maintained under somewhat unnatural conditions (in chambers). The proximity of other individuals could have depressed growth and maturation rates, or increased mortality. Alternatively, a predictable food source may have enhanced growth and extended lifetimes, although this is doubtful. Field data, however, indicate an 8-month interval during which the mean and maximum sizes of males and females increased. This may represent the collective growth of a large group of individuals, perhaps a cohort produced by spring reproduction. The period of growth was followed by a 4-month interval in which mean male and female sizes, and numbers of large individuals, decreased. The rapid decrease in mean sizes could have resulted from recruitment of small individuals produced by fall reproduction alone, but the loss of large individuals indicates mortality. A longer study could elucidate a precise figure for longevity in this species, but it seems safe to state that most individuals live about 8 months or less, and certainly less than 1 year.

While confinement may have affected the longevity and overall growth rates of lab-reared individuals, the *relative* growth rates of males and females are not likely to have been significantly altered. Males grew more rapidly and reached sexual maturity sooner than females. Since male-male competition in this species is high, and since large size confers a reproductive advantage to males, the selective context for rapid growth and maturation rates among males is clear.

Hermaphroditism and sex-change have been reported in certain isopods (Cymothoidae; Richardson, 1905), but neither condition was observed in this species. Both hermaphroditism and sex-change are restricted primarily to parasitic species, or species with a history of reduced effective population size and/or highly skewed operational sex ratios (Ghiselin 1974). Such conditions are unlikely for *T. thermophilum*, or for that matter, for any other free-living, freshwater isopod species.

Female reproductive strategies in this species are likely to be complex given the array of female attributes that seem related to variable reproductive effort. Iteroparity and the ability to resorb developing young certainly indicate that females can reallocate reproductive resources when conditions (environmental and physiological) become unfavorable (see Lawlor 1976). Females produce larger broods as they age and grow, a relationship usually explained as a consequence of increased ovary and marsupium size, and one that implies selection for large females. Females in this species, however, are surprisingly small. In addition, variability in female size is low, and is considerably less than variability in male size. Downhower (1976) has indicated that in finches (Geospizinae), selection favors small females in habitats where food is scarce because small females accumulate sufficient resources for reproduction faster and more frequently than large females. If small female size is favored in *T. thermophilum*, females may place less energy into growth than other isopod species (Lawlor 1976) and may reproduce relatively continuously rather than seasonally. Although this seems to fit the data presented here, information on resource availability in Sedillo Spring, and on relationships between growth, energy storage and reproductive patterns in females are necessary to determine if the hypothesis is appropriate.

ACKNOWLEDGMENTS

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LITERATURE CITED

ALLEE, W. C. 1914. The ecological importance of the rheotactic reaction of stream isopods. *Biol. Bull.* **27:** 52–66.

ALLEE, W. C. 1927. Animal aggregations. *Q. Rev. Biol.* **2:** 367–398.

BRUES, C. T. 1932. Further studies on the fauna of North American hot springs. *Amer. Acad. Arts Sci.* **67**(7): 185–303.

COLE, G. A., AND C. A. BANE. 1978. *Thermosphaeroma subequalum,* n. gen., n. sp. (Crustacea: Isopoda) from Big Bend National Park, Texas. *Hydrobiologia* **59:** 223–228.

DOWNHOWER, J. F. 1976. Darwin's finches and the evolution of sexual dimorphism in body size. *Nature* **263:** 558–563.

ELLIS, R. S. 1961. A life history of *Asellus intermedius. Trans. Amer. Microsc. Soc.* **80:** 80–102.

FEDERAL REGISTER. 1977. Vol. 42, No. 251, 50 CFR Part 17. Pp. 65213–65214.

GHISELIN, M. T. 1974. *The economy of nature and the evolution of sex.* Berkeley, University of California Press. Pp 108–124.

GWYNNE, D. T., AND K. M. O'NEILL. 1980. Territoriality in digger wasps results in sex biased predation on males (Hymenoptera: Sphecidae, *Philanthus*). *J. Kans. Ent. Soc.* **53:** 220–224.

HANSEN, H. J. 1906. On the propagation, structure and classification of the family Sphaeromatidae. *Q. J. Microsc. Sci.* **49:** 69–134.

HATCHETT, S. P. 1947. Biology of the Isopoda of Michigan. *Ecol. Monog.* **17:** 47–79.

LAWLOR, L. W. 1976. Molting, growth and reproductive strategies in the terrestrial isopod, *Amadillidium vulgare. Ecology* **57:** 1179–1194.

MAGNIEZ, G. 1978. Précopulation et vie souterraine chez quelques Péracarides (Crustacea: Malacostraca). *Arch. Zool. Exp. Gen.* **119:** 471–478.

MANNING, J. T. 1975. Male discrimination and investment in *Asellus aquaticus* (L.) and *A. meridianus* Racovitsza (Crustacea: Isopoda). *Behaviour* **55:** 1–14.

MARKUS, H. C. 1930. Studies on the morphology and life history of the isopod, *Mancasellus. J. Linn. Soc.* **34:** 334–343.

PARKER, G. A. 1974. Courtship persistence and female guarding as male time investment strategies. *Behaviour* **48:** 157–184.

RICHARDSON, H. 1897. Description of a new crustacean of the genus *Sphaeroma* from a warm spring in New Mexico. *Proc. U. S. Nat. Mus.* **20:** 465–466.

RICHARDSON, H. 1900. Synopsis of North American invertebrates. VIII. The Isopoda. *Am. Nat.* **34:** 207–230.

RICHARDSON, H. 1904. Contributions to the natural history of the Isopoda. *Proc. U. S. Nat. Mus.* **27:** 1–89.

RICHARDSON, H. 1905. A monograph on the isopods of North America. *Bull. U. S. Nat. Mus.* **54:** 1–727.

RIDLEY, M., AND D. J. THOMPSON. 1979. Size and mating in *Asellus aquaticus* (Crustacea: Isopoda). *Z. Tierpsychol.* **51:** 380–397.

RIOJA, E. 1950. Estudios Carcinologicos XXIII. Estudio morfologico del *Exosphaeroma dugesi* (Dollfus), Isopoda termofilo de la fauna Mexicana. *Ann. Inst. Biol. Mex.* **21:** 351–365.

SHUSTER, S. M. 1979. Aspects of the life history and reproductive biology of the Socorro Isopod, *Thermosphaeroma thermophilum* (Crustacea: Peracarida). Unpublished M.S. Thesis, the University of New Mexico. 93 pp.

SHUSTER, S. M. 1981. Sexual selection in the Socorro Isopod, *Thermosphaeroma thermophilum* (Cole) (Crustacea: Peracarida). *Anim. Behav.* **29:** 698–707.

THORNHILL, R. 1977. The comparative predatory and sexual behavior of hangingflies (Mecoptera: Bittacidae). *Occ. Papers Mus. Zool. Univ. Mich.* **677:** 1–43.

THORNHILL, R. 1978. Sexually selected predatory and mating behavior of the hangingfly, *Bittacus stigmaterus* (Mecoptera: Bittacidae). *Ann Entomol. Soc. Am.* **71:** 597–601.

THORNHILL, R. 1979. Male and female sexual selection and the evolution of mating strategies in insects. Pp. 18–121 In M. Blum, Ed., *Sexual selection and reproductive competition.* New York, Academis Press.

TRIVERS, R. L. 1972. Parental investment and sexual selection. Pp. 136–177 in B. Campbell, Ed., *Sexual selection and the descent of man: 1871–1971.* Chicago, Aldine-Atherton.

VAN NAME, W. G. 1936. The American land and fresh-water isopod Crustacea. *Bull. Amer. Mus. Nat. Hist.* **71.**

VEUILLE, M. AND J. ROUAULT. 1980. Experimental evidence of sexual selection based on male body size in *Jaera* (Isopoda: Asellota). *Experientia* **36:** 549–550.

ZAR, J. 1974. *Biostatistical analysis.* Prentice Hall, Englewood Cliffs, N.J. Pp. 108–109.

Reference: *Biol. Bull.* **161**:303–355. (October, 1981)

ABSTRACTS OF PAPERS PRESENTED AT THE GENERAL SCIENTIFIC MEETINGS OF THE MARINE BIOLOGICAL LABORATORY

AUGUST 18–21, 1981

Abstracts are arranged alphabetically by first author within the following categories: Cell motility and cytoskeleton, comparative physiology and biochemistry, developmental biology, ecology, fertilization and reproduction, marine resources, neurobiology, and parasitology. Author and subject references will be found in the regular volume index in the December issue.

CELL MOTILITY AND CYTOSKELETON

Fast axonal transport in the squid giant axon. ROBERT D. ALLEN (Dartmouth College), JANIS METUZALS, AND ICHIJI TASAKI.

Up to now, the assumption that synaptic vesicles and larger organelles are transported along squid giant axons had not been confirmed by direct observation.

We now report massive bidirectional movements of submicroscopic particles at a velocity of 1–2 μm/sec, made visible by AVEC-DIC video-enhanced microscopy (Allen *et al.* 1981, *Cell Motility* **1**: 291–302) along linear structures in the axoplasm. Movements persisted for at least 30 min after the nerves were excised, and were observed throughout the cortical and endoplasmic regions in the raw video image. The images could be enhanced by either time-averaging the video signal over 0.25 sec or by subtracting the background, out-of-focus image using a Hamamatsu frame memory with the C-1000 Chalnicon camera.

We interpret the observed motions as the fast axonal transport of synaptic vesicles and other structures along either neurofilaments or (more likely) microtubules. The present observations confirm those obtained with chick spinal-ganglion cells, where similar continuous motions of submicroscopic particles (in addition to saltatory motions of organelles) have been described (Breuer *et al.* 1981, *Neurology* **31**: 118a). Similar movements have been reported in a variety of other eukaryotic cells (Allen *et al.* 1981, Cold Spring Harbor Symposium on the Cytoskeleton, in press).

Molecular components of anucleate cytoskeletons prepared from dogfish erythrocytes. DIANA C. BARTELT AND WILLIAM D. COHEN (Hunter College, N. Y.).

The cytoskeletal system of dogfish (*M. canis*) erythrocytes was studied to elucidate the protein composition of its structural components and the possible role of calcium and calmodulin (CaM) in cytoskeleton formation and function. To simplify analysis, a method was developed for preparing anucleate ghosts in quantity from these nucleated cells. Treatment of the ghosts with Triton X-100 under microtubule-stabilizing conditions yielded anucleate cytoskeletons, most of which retained the flattened elliptical morphology typical of the living cells. The anucleate cytoskeletons consisted of a network of material with a marginal band of microtubules enclosed within, as seen in uranyl acetate-stained whole mounts (TEM). The major protein components of the anucleate cytoskeletons were spectrin, actin, and tubulin as judged by comigration with standards during polyacrylamide gel electrophoresis in SDS (SDS-PAGE). Also present were proteins whose migration corresponded to goblin and vimentin, as reported by others for avian erythrocytes. In contrast, marginal bands isolated from nucleated cytoskeletons contained only tubulin as a major component; spectrin and actin were absent. Therefore, spectrin and actin appear to be localized in the network material, with tubulin in the marginal band. As we have shown previously, calcium and ionophore A23187 cause calcium-dependent changes in dogfish erythrocyte morphology. We wished to determine whether CaM could be a mediator of these effects. CaM was detected in dogfish erythrocyte lysates at a minimal concentration of 2μM. CaM-binding proteins were detected by means of ^{125}I CaM binding to cytoskeletal proteins separated by SDS-PAGE. Subsequent autoradiography revealed one major calcium-dependent CaM-binding protein (CBP), with an apparent molecular weight of 245,000. CBP comigrated with the upper band of dogfish erythrocyte spectrin. Neither band of human erythrocyte spectrin bound CaM in this assay. These results are consistent with a possible role for calmodulin in the mediation of calcium effects on nucleated erythrocyte morphology.

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Fast axonal transport in extruded axoplasm from squid giant axon. SCOTT T. BRADY (Dept. of Anatomy, Case Western Reserve Univ.), RAYMOND J. LASEK, AND ROBERT D. ALLEN.

To understand the molecular mechanisms of axonal transport, model systems are required that permit direct analysis of the moving elements and that are freely accessible to biochemical and pharmacological manipulation. For fast axonal transport, this requires detection of moving vesicles as small as 30–50 nm and the maintenance of movements in the absence of permeability barriers. Previously available preparations have been unable to fully satisfy either of these criteria.

To obtain axoplasm free of permeability barriers, the cytoplasmic contents of the squid giant axon can be mechanically extruded, free of its plasma membrane and connective tissue sheath, onto a cover slip. Axoplasm extruded in this manner maintains its metabolic activity and structural organization. The advent of video enhanced light microscopic techniques (AVEC-DIC) capable of detecting structures as small as 25 nm (Allen *et al.* 1981, *Cell Motility* **1**: 291–302) has permitted for the first time analysis of the axonal transport processes in extruded axoplasm.

Initial results with this model indicate at least three size classes of particles moving in extruded axoplasm: (1) small rapidly moving particles that make long traverses in the anterograde direction, (2) medium sized elongate or spheroidal particles that can be seen to proceed in both retrograde and anterograde directions, and (3) large, slower moving particles that move in both directions. The large particles have been identified as mitochondria, both because they are the only common structures of comparable size in ultrastructural studies of axoplasm, and because treatment of axoplasm with 2,6 dinitrophenol (200 μM) in the presence of ATP (1 mM) selectively alters the shape and inhibits the movement of the large particles, while DNP in the absence of ATP inhibits movement of all three classes. Particle movement has been maintained for more than an hour with repeated perfusions of appropriate buffers. In extruded axoplasm, particles continue to move along linear pathways defined by fibrillar elements. Particle movement continues even when the organization of the fibrillar elements has been disrupted by stirring. In the disrupted axoplasm, particles move both along fibrillar elements and between them.

Our studies indicate that the extruded axoplasm model viewed in this way is an ideal system for studying the molecular mechanisms of intracellular transport and cell motility.

Secretory granules are attached to microtubules released from the endocrine pancreas. WILLIAM L. DENTLER (Department of Physiology and Cell Biology, University of Kansas, Lawrence, KS).

Previous studies have shown that filamentous bridges link microtubules with endocrine pancreatic secretory granules *in situ* and that high molecular weight microtubule-associated proteins (MAPs), purified from avian brain, can link these secretory granules to microtubules *in vitro* (Suprenant and Dentler, *J. Cell Biol.*, in press). To directly study associations between microtubules and secretory granules, the endocrine pancreas from the anglerfish was homogenized in microtubule-stabilizing solutions that contained 100 mM MES, pH 6.4, 2 mM EDTA, 2 mM EGTA, 1 mM $MgSO_4$, 250 mM sucrose, 2 mM DTT, 1 mM GTP, 100 μg/ml antipain and leupeptin, and either 1 M hexylene glycol or 10 μM Taxol to stabilize microtubules. Homogenates were centrifuged for 5 min at 600 × *g*, and the supernatants were centrifuged for 20 min at 20,000 × *g* at 4°. The high speed pellets were resuspended in homogenization buffer, were fixed by dilution with 1% glutaraldehyde, 0.1% tannic acid, and 100 mM phosphate buffer, and were imbedded in plastic. Thin sections of this material revealed secretory granules, mitochondria, microtubules, and microsomes. Virtually all of the microtubules had one or more secretory granules attached to them by filaments similar in appearance to the MAPs. Secretory granules were the only organelles observed to be attached to the microtubules. Preliminary analysis of the microtubule proteins was carried out by resuspending the high speed pellets in buffer without microtubule-stabilizing agents and examining by electrophoresis the proteins released upon microtubule depolymerization. Both tubulin and a protein that co-migrated with MAP-2 were present in the preparations although the presence of 15–20 other proteins bands that migrated between 55,000 and 400,000 daltons prevented the specific identification of microtubule-associated proteins. These results clearly demonstrate that microtubules and secretory granules are linked in the endocrine pancreas by filamentous MAP-like structures and that the complexes are sufficiently strong to permit their eventual isolation and biochemical analysis.

Analysis of the mobility of a soluble enzyme in squid axoplasm. KARL R. FATH (Case Western Reserve University), JAMES R. MORRIS, AND RAYMOND J. LASEK

The solubility of phosphoglucoisomerase (EC 5.3.1.9) in the giant axon of *Loligo pealei* was studied. Axoplasm was separated from its surrounding membrane by extrusion into a 0.7 ml buffer that simulates the solution conditions in the axon. Soluble proteins elute out of the axoplasm into the bath and equal samples were removed at selected times. The samples were analyzed for PGI activity using a standard coupling assay with glucose 6-phosphate dehydrogenase by spectrophotometrically monitoring the reduction of NADP at 340 nm.

Diffusion theory predicts an exponential course of elution of monomeric PGI from the axoplasmic cylinder. Actual PGI activity elutes more slowly than predicted by theory. On a semi-log plot, PGI elution can be modeled as a sum of two exponentials. There is an early, rapid component that lasts for 20 min and a slower component that extends until 120 min. All PGI in the axoplasm has eluted by the end of the second component. The rates of both components differ by as much as an order of magnitude. The rate of the first component can be explained by free diffusion of monomeric PGI. Isokinetic sucrose gradients of fractions of both components as well as from whole axoplasm suggest that PGI is not complexed with other proteins as it elutes from axoplasm. We propose that elution of some of the PGI may be delayed due to associations with non-diffusing structures in the axoplasmic cylinder. Electron microscopic and axonal transport studies that demonstrate non-random movements of soluble enzymes in association with cytoskeletal structures support this hypothesis.

Taxol-induced redistribution of microtubules (MT) and intermediate filaments (IF) in cultured cells. KATHLEEN J. GREEN AND ROBERT D. GOLDMAN (Northwestern University Medical School).

It has been suggested that a functional association exists between microtubules (MT) and intermediate filaments (IF) in several cell types. This association can be disrupted by colchicine, which depolymerizes MT and causes the formation of a juxtanuclear "cap" of IF. Taxol increases the rate and extent of tubulin polymerization and stabilizes MT *in vitro* (Schiff *et al.* 1979, *Nature.* **277:** 665–667). We have investigated taxol's effects on ENSON cells (human skin fibroblasts) and BHK-21 cells in culture.

Cells grown on coverslips were treated with 1–10 μM taxol for 1–24 h. As seen by phase contrast microscopy, taxol induces changes that mimic those produced by colchicine. These include a loss of major cellular extensions, a redistribution of organelles to a more perinuclear location, and an inhibition of saltatory movements and locomotion. Ruffling and filopod formation continue, indicating that cells are viable up to 24 h. Polarized light microscopy reveals perinuclear birefringent material in taxol treated cells. This material was examined by indirect immunofluorescence using antisera directed against 6S bovine brain tubulin or BHK-21, 55,000 dalton, IF protein. In control cells, IF and MT appear to radiate from a single juxtanuclear focal point, and extend to the cell periphery. In taxol treated cells, MT and IF are excluded from the cell margins and are found in large complexes in the same area as the birefringent material. Ultrastructural analysis reveals that in taxol treated cells MT form bundles within which IF interdigitate, indicating that an MT-IF association may be retained. These results indicate that maintenance of normal cell shape and motility is dependent upon a carefully regulated dynamic state of IF-MT interactions, which can be perturbed by agents which either depolymerize or stabilize MT *in vivo.*

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Immunological localization of an intermediate filament-like protein in Arbacia *sperm flagella.* GORDON E. HERING (University of Wisconsin, Madison) AND ROGER D. SLOBODA.

It has been reported (Linck *et al.* 1981, *Biophys. J.* **33:** 215a) that intermediate filament protein exists as a structural entity in flagellar outer doublet microtubules. To test this, antibodies directed against the 55K dalton polypeptide of intermediate filaments of BHK cells were used for indirect immunofluorescence and ^{125}I-Protein A staining of SDS-gels containing various fractions of flagellar proteins. When either intact sperm or isolated axonemes were used for indirect immunofluorescence, staining was observed along the entire length of the sperm tail and axoneme, along with a small but distinct area of fluorescence coincident with the acrosome in samples of intact sperm. Control experiments in which BSA was used in place of the primary antibody, or in which fluorescent goat anti-rabbit antibody was used alone, did stain either whole sperm or isolated axonemes. To determine which protein of the flagellum was responsible for the immunofluorescence results, whole flagella were separated into their protein components by SDS-polyacrylamide gel electrophoresis; the gel was then incubated in antibody, and then in iodinated Protein A, dried, and exposed to X-ray film. The results showed that the antibody bound specifically to a band on the gel having a molecular weight of approximately 35K daltons. These results show, first, that an antigen recognized by antibodies to BHK intermediate filament protein resides in the sperm flagellum, and second, that the protein responsible has a molecular weight considerably less than the native antigen to which the antibody is directed. These results suggest either that proteolysis has broken down the 55K antigen into α-helical and globular regions or that the flagellum of *Arbacia* contains a protein whose native molecular weight is normally less than the 55K protein of BHK cells, but still contains the same antigenic determinants as 55K intermediate filament protein.

We thank Robert Goldman for generously supplying the antibodies and acknowledge Joel Rosenbaum and Emilio Stromboli for many stimulating discussions. This study was performed in the Physiology Course at MBL and was supported by USPHS Training Grant GM07784.

High resolution, stereo video microscope. SHINYA INOUÉ (MBL Woods Hole, MA), DAVID COHEN, AND GORDON W. ELLIS.

We report here a video enhanced light microscope that produces three-dimensional images at high resolution. Rapidly changing objects can be examined since the specimen need neither be tilted nor displaced. Instead, the stereo image pair is formed by light that travels obliquely through a high numerical aperture (N.A.) objective lens. The beams are effectively tilted by alternately masking parts of the objective lens back aperture, or its conjugates, with a sector wheel. The wheel is driven, in synchrony with the successive fields of the video camera, by a video processor phase-locked to the camera. The two outputs of the processor alternately provide the left and right video fields. On a single monitor, the left and right image fields can be alternately blanked with electro-optically shuttered goggles. Or, on two monitors coupled with a beam splitter, they can be alternately blanked by complementary polarizing or colored glasses.

Alternatively, a stationary, complementary colored half-shade filter can be placed at the objective-lens back aperture or its conjugate planes. The tilted, colored beams provide the left and right images on a color video camera. The display on a color video monitor is viewed through complementary colored glasses. This method is simpler but cannot produce color stereo images.

A monochrome camera with automatic contrast and black level controls, combined with a sector wheel placed at the microscope eyepoint, gave good, high resolution stereo images of diatoms in birefringence and differential-interference-contrast modes with an 0.95 N.A. Plan Apochromatic objective lens. A red-green half-shade filter placed next to the condenser iris diaphragm gave good stereo images of diatom shells and of the helical path of swimming *Arbacia* and *Chaetopterus* sperm through a color video camera and monitor. Supported by NIH grant 5RO-1 GM 23475-16 and NSF grant PCM 7922136.

Cyclic birefringence changes in Physarum *plasmodium.* NOBURÔ KAMIYA AND ROBERT D. ALLEN (Dartmouth College).

The behavior of birefringent fibris (BRF) in *Physarum* plasmodium was studied with special reference to their relation to cytoplasmic streaming with AVEC-POL microscopy (Allen *et al.* 1981, *Cell Motility* **1:** 275–289). The preparations were small plasmodia spread into a thin sheet between coverslip and agar film. In the narrow zone behind the leading edge of an advancing plasmodium, more BRF appeared when the endoplasm flowed away from the front, or in the emptying phase (Kamiya 1973, *Proc. 4th Int. Biophys. Congr. Moscow, Symp. III* 447–465). When the fan-like expanse approached its maximum contraction phase and streaming began toward the front (filling phase), most of the BRF disappeared, except for a few that formed "knots" in the zone several tens of microns behind the front. The knots were left behind and eventually dissolved as the front advanced. At the same time, new knots emerged in the new frontal area. When the fan-like expanse ceased to move forward, the BRF tended to persist even in the filling phase. BRF far behind the front zone, *e.g.* ring-like BRF surrounding veins at right angles to their axes, were much more stable; their birefringence was strengthened when strands became thicker and weakened when they became thinner, suggesting strain birefringence (Nakajima and Allen 1965, *J. Cell Biol.* **25**: 361–374). The plasmodium advanced only when the BRF in the front zone disappeared, suggesting the view of Yoshimoto and Kamiya (1978, *Protoplasma* **95**: 123–133) that the forward streaming is caused by tonic contraction at the rear. The sites where BRF appear coincide with the areas where contraction occurs in reactivated glycerinated models (Kamiya and Kuroda 1965, *Proc. Jpn. Acad.* **41**: 837–841). Further, changes in birefringence throughout emptying-filling cycles of the advancing front find their exact counterpart in the filament cycle model elaborated by electron microscopy (Nagai *et al.* 1978, *J. Cell Sci.* **33**: 121–136).

Effects of increasing deuterium oxide concentrations on meiotic spindle formation in Chaetopterus pergamentaceous *oocytes.* DOUGLAS A. LUTZ AND SHINYA INOUÉ (Marine Biological Laboratory, Woods Hole, MA).

Germinal vesicle (GV) stage oocytes released into sea water undergo a series of events resulting in a metaphase arrested spindle attached to the animal pole cortex. Sequential events of normal spindle formation, examined with DIC and polarized light microscopy, are: GV breakdown, convergence of centrosomes, migration to the cortex, and maturation of the spindle. Oocytes shed directly into concentrations ≤35% D_2O in sea water manifest this sequence, but the time required for the events increases slightly with increasing D_2O concentrations. Concentrations of 40–45% D_2O allow GV breakdown and centrosome convergence but inhibit spindle migration to the cortex. Spindles so formed, more birefringent than controls, remain in the area of centrosome convergence. Replacing D_2O with normal sea water initiates spindle migration. In oocytes shed into 50% D_2O, spindle formation is not observed, although GV breakdown occurs. Oocytes thus remain frozen at this stage. Normal, sequential spindle formation events resume after D_2O is replaced with normal sea water. Concentrations of 40 and 50% D_2O applied to fertilized eggs before first cleavage metaphase do not inhibit spindle function, including anaphase chromosome movement. Inhibition of spindle migration to the cortex during immersion in 40–45% D_2O may result from: (1) counteraction of a local microtubule-depolymerization mechanism prerequisite for movement, (2) gelation of the cytosol, or (3) inhibition of a cortical-spindle interaction necessary for directed movement. Effects of 50% D_2O may be analagous to the D_2O mitotic inhibition thought to be due to D-substitution for weaker H-bonding (Gross and Spindel 1960, *Ann. N. Y. Acad. Sci.* **84**: 745–754).

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A theoretical model for the action of clathrin and ion gradients in receptor-mediated endocytosis. DANA NOEL MCKINLEY (Dept. of Biochemistry, University of North Carolina, Chapel Hill).

The energetics of the invagination step in receptor-mediated endocytosis was analyzed and models presented. It is assumed that dispersed receptors bind ligand, inducing a phase separation into a patch to which clathrin triskelions bind in a hexagonal lattice. I propose that there is a large release of free energy upon assembly; and that triskelions are energetically equally liable to pair in a shape conducive to pentagons or hexagons, but are constrained to assemble as hexagons on a flat membrane. A high activation energy, provided by thermal motion, bends the patch inward and allows a self-propagating cooperative lattice rearrangement. This rearrangement leaves some pentagons, stabilizes curvature, and enormously increases the statistical mechanical probability of the lattice arrangement. Work against the surface tension of the surrounding membrane (estimated as 500 kcal) would be provided by the consolidation of the potential clathrin-clathrin bonds around the free edge as the disk becomes a sphere. The stable clathrin cage would require energy, perhaps via phosphorylation, for disassembly. Subsequent cleavage of phosphate would leave free triskelions ready for reassembly catalyzed by receptor patches. This is essentially different from the long-standing model that hexagon-pentagon transition is forced and "powers" infolding.

Alternatively, I propose that receptor patches themselves transduce energy from ion gradients across organelle membranes for endocytosis. For instance, ligand binding and clustering might make the membrane leaky and allow ligand-induced calcium binding on both surfaces at a high local calcium concentration, thereby favoring a receptor-mediated destabilization of patch-membrane interaction, allowing spontaneous endocytosis. Vesicles then would lose calcium to cytoplasm, restoring receptors.

Swelling of golgi (and lysosomes) treated with ionophores, and concomitant interference with vesicle transfer (Strous and Lodish 1980, *Cell* **22**: 709–717) indicate that internal membrane compartments that exchange with coated vesicles contain the required ion pumps.

Modes of crosslinking in neurofilament protein isolated from squid giant axon: electron microscopic evidence for paracrystalline arrays. JANIS METUZALS AND DAVID F. CLAPIN (Faculty of Health Sciences, University of Ottawa, Ottawa Ontario, KIN 9A9, Canada.

It has been demonstrated that the two major neurofilament peptides of squid axoplasm (60 and 200 K) copurify with a high molecular weight peptide (~380 K). Little is known about the role of this peptide in the neurofilamentous network. We report a procedure for the isolation of this peptide from homogenates of squid axoplasm and present electron microscopic data concerning its morphology.

Axoplasm from 14 axons was extruded into a solution containing 0.15 *M* KCl, 5 m*M* EGTA, 1 m*M* 2-mercaptoethanol, 10 m*M* Tris-HCl, pH 7.4 and was homogenized in a glass-teflon homogenizer at 0°C. The homogenate was centrifuged for 15 min at 25,000 × *g* at 4°C. The supernatant was applied to a 0.9 × 59 cm column of 4% agarose (Bio-Gel A-15 m) equilibrated with the homogenizing buffer and operated at 4°C. A protein peak eluting immediately after the void volume was found to contain a high molecular weight peptide as determined by SDS-PAGE on 5% gels. Its molecular weight was estimated to be ~380,000 daltons. This peptide co-migrated with a high molecular weight peptide of whole axoplasm known as NF-1. Samples of eluate containing this peptide were placed on coated grids and stained for 3 min with 2% uranyl acetate.

Sheets of filaments 2 to 5 nm in diameter forming network arrays, which in some areas appear as regular hexagons 20 nm long at each edge, are observed. These fine filaments are connected with 10 nm neurofilaments, giving the latter a lampbrush-like appearance. The intact neurofilaments display a distinct helical substructure consisting of four intercoiled 2-nm-wide "unit filaments" and periodically distributed pairs of sidearms oriented at various angles.

We conclude that the high molecular weight neurofilament peptide forms 5-nm-wide filaments which interconnect the 10-nm-wide neurofilaments to form the neurofilamentous network.

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The microtubule network in the squid giant axon. JAMES R. MORRIS (Center for Cancer Research, MIT), ALAN J. HODGE, AND RAYMOND J. LASEK.

The squid giant axon contains both microtubules (MT) and neurofilaments (NF) in its cytoskeleton. These fibrillar polymers support the axon's cylindrical shape by forming networks which include cross-linking elements. We now report that the MT network in the giant axon can be purified without significantly disturbing its organization *in vivo.* A putative MAP is included as a major component in the preparation. Furthermore, this protein appears to be an important cross-linking element in the MT network.

Axoplasm can be removed from its sheath by extruding it directly into a buffer of low chaotropicity. Extruded axoplasm retains the cylindrical form of the axon for more than 24 h. The NF network of the axon is insoluble, but most of the tubulin and actin is extracted from the axoplasm. The extracted axoplasmic cylinder contains a stable network; it may be lifted out of the bath with forceps without producing changes in its shape.

Five micromolar taxol stabilizes the MT in extruded axoplasm so that the tubulin and a putative MAP of about 300k daltons are retained in the insoluble cytoskeleton. The neurofilaments can be removed from this network with 5 m*M* calcium because of a calcium-activated protease that remains associated with the cytoskeleton. This preparation retains its network properties after calcium treatment. SDS-PAGE reveals that all of the NF proteins are degraded, but the tubulin and the putative MAP are apparently unaffected. Electron microscopy reveals that the NF network is lost and an array of MT fills the cylinder. Mitochondria and small vesicles also remain, suggesting that they are associated with the MT. These results lead to the conclusion that the MT in the giant axon interact to form a network in the absence of NF. The network properties are lost when this preparation is treated with trypsin, which selectively cleaves the putative MAP but not the tubulin or MT. We suggest that a MAP cross-links MT and stabilizes the MT network in the axon.

Properties of the unilateral ciliary reversal response during prey capture by Pleurobrachia *(Ctenophora).* ANTHONY G. MOSS AND SIDNEY L. TAMM (Boston University Marine Program).

Ciliary responses involved in the feeding behavior of the ctenophore *Pleurobrachia pileus* were studied by high-speed cinemicrography. *Pleurobrachia* fishes with its two long tentacles outstretched into a net. Capture of small zooplankton by either tentacle causes independent contractions of the tentacles. The ctenophore then dashes forward at high speed for ~3 sec, due to increased frequency of beating ($\sim$11 Hz) on all comb rows. A brief arrest ($\sim$0.5 sec), followed by active flattening of the plates against the row, typically occurs in the four comb rows on the side of the prey-catching tentacle.

The *Pleurobrachia* then spins rapidly 2–3 times in the tentacular plane toward the food-carrying tentacle, sweeping it into the mouth, which bends toward it. Spinning is caused by a localized reversal of beat direction on the prey-catching side. Ciliary reversal occurs on both the two subtentacular and the two subsagittal rows of the prey-catching side in 52% of cases ($n = 203$; 31 animals), but only on the subtentacular rows 48% of the time. In the latter cases, the subsaggital rows usually remain quiescent, or rarely beat in the normal direction. Reversed beating occurs at twice the beat frequency (*i.e.*, 21 Hz) of the normal side.

Reversed beating is initiated at the oral end of the row by a recovery stroke directed aborally. Neighboring plates, successively lifted in the aboral direction, perform an effective stroke toward the mouth. Metachronal waves are thus propagated in the opposite direction during reversal, maintaining antiplectic coordination.

The stimulus for beginning reversed beating, and the mode of propagation of reversed waves, were investigated by pressing thin plastic strips midway along the subtentacular rows before the onset of reversal. Such mechanical blocks interrupt propagation of normal waves, and prevent transmission of high beat frequency during the fast forward-swimming preceding reversal. In contrast, although waves of reversed beating never passed across the block, reversed waves were almost always initiated independently at the aboral side of the block, usually before the reversed wave from the oral end reached the block. The block itself thus acted as a stimulus to initiate reversed beating, as only occasionally happens during normal beating. The frequency of reversed beating on the aboral side was slightly less (19 Hz) than that on the oral side (23 Hz).

Thus, a heightened level of mechanosensitivity of the comb plates is important in initiating and propagating reversed waves. In addition, a "priming stimulus" for high frequency reversal is evidently conducted from a tentacle to all plates along the 4 rows on that side.

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The cytoskeleton of the Lytechinus pictus *egg before and after fertilization.* ROBERTO F. NICOSIA (Medical College of Pennsylvania, Philadelphia, PA), WILLIAM R. JEFFERY, BRUCE BRANDHORST, AND RANDALL T. MOON.

The structural organization of the cytoskeleton of detergent-extracted *Lytechinus pictus* eggs was studied by scanning electron microscopy (SEM) and polyacrylamide gel electrophoresis (PAGE). Unfertilized or fertilized eggs, with extracellular membranes removed, were extracted for 1 h with 100 m*M* KCl, 5 m*M* Mg acetate, 20 m*M* MOPS, 300 m*M* sucrose, 5 m*M* EDTA (or 5 m*M* EGTA), 0.5% Triton X-100, and 5 m*M* phenylmethylsulfonylfluoride. The pH of the extraction buffer was 6.7 for unfertilized eggs and 7.3 for fertilized eggs. For SEM, the extracted cytoskeletons were fixed in 2% glutaraldehyde, post-fixed in 0.5% OsO_4, dehydrated in ethanol, and critical-point dried.

The surface of the cytoskeleton of unfertilized eggs was characterized by a uniform pattern of spherical granules about 1 μ in diameter. They were often arranged in interlaced rows and appeared to be connected to an underlying filamentous network. These spherical entities are probably cortical granules, since they are disrupted by treatment with 10^{-3} M Ca^{2+}. The surface of the cytoskeleton of fertilized eggs lacked the cortical granules but contained a thick meshwork of filaments extending for a depth of 6–7 microns into the cytoskeleton. PAGE showed that cytoskeletons were enriched in 68K, 67K, 62K, 59K, 56K, 53K, 50K, 43K, 33K, and 30K polypeptides. No qualitative differences were seen in the cytoskeletons of fertilized and unfertilized eggs. The 43K protein, which co-migrated with actin, was enriched in the cytoskeleton when EGTA rather than EDTA was in the extraction buffer. Since the filamentous meshwork was observed even in the absence of the putative actin component, we conclude that other detergent-insoluble proteins are major components of the cytoskeleton of sea urchin eggs.

Iontophoretic injection of vanadate (V^V) *anion inhibits chromosome movement at micromolar concentrations.* R. E. STEPHENS, E. W. STOMMEL, AND S. INOUÉ (Marine Biological Laboratory).

Dynein has been implicated in chromosome movement from its presence in isolated spindles and from the inhibition of chromosome movement in reactivated cell models by vanadate (V^V) anion, a potent inhibitor of ciliary and flagellar dynein cross-bridging. Iontophoretic injection of vanadate to levels of about 10 μM stops ciliary movement in hair cells of the nudibranch *Hermissenda.* This same concentration stops ciliary movement when applied to reactivated cell models of *Paramecium.* In preliminary experiments, occasional stoppage of chromosome movement in echinoderm eggs occurs at 100 μM levels of injected vanadate. Spectrophotometrically, we find that sulfhydryl agents (reduced glutathione, mercaptoethanol, dithiothreitol) reduce vanadate (V^{V}) to (V^{IV}), an oxidation state ineffective in inhibiting dynein ATPase. Considering the millimolar levels of reduced glutathione (GSH) in echinoderm eggs, the resultant concentration of vanadate (V^{V}) must be considerably lower than that injected. We subsequently reduced the level of GSH through incubating fertilized eggs in methyl phenylazoformate at 10 μM, a concentration that allows normal development through gastrulation. In this medium, injection of vanadate to 10 μM levels inhibits anaphase chromosome movement but permits normal cytokinesis. Injection at prometaphase prevents chromosome congression; using a two-cell embryo such a cell remains suspended in time while the non-injected sister cell divides. Application of norepinephrine, which reduces V^{V} to V^{IV}, results in metaphase alignment, chromosome separation, movement, and cytokinesis. These results indicate the involvement of a vanadate-sensitive system in chromosome movement; dynein-based tubule sliding or a Ca^{++}-ATPase pump are possible candidates.

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Specific localization of scallop gill epithelial calmodulin in the ciliary matrix.

Elijah Stommel, Ray Stephens (Marine Biological Laboratory), Robert Masure and James Head .

Calmodulin has been isolated and characterized from the gill of the bay scallop *Aequipecten irradians.* Quantitative electrophoretic analysis of epithelial cell fractions shows most of the calmodulin to be localized in the cilia, specifically in the detergent-solubilized membrane-matrix fraction. Calmodulin is 2.2 ± 0.3% of the membrane-matrix protein or 0.41 ± 0.05% of the total ciliary protein. Its concentration is at least 10^{-4} *M*, if it is distributed uniformly within the matrix. Extraction in the presence of calcium suggests that the calmodulin is not bound to the axoneme proper. The ciliary protein is identified as a calmodulin on the basis of its calcium-dependent binding to a fluphenazine affinity column and its comigration with bovine brain calmodulin on alkaline-urea and SDS polyacrylamide gels in both the presence and absence of calcium. Scallop ciliary calmodulin activates bovine brain phosphodiesterase to the same extent as bovine-brain and chicken-gizzard calmodulins. Lacking cysteine and tryptophan, the amino acid composition of gill calmodulin is typical of known calmodulins, with the exception of an unusally high serine content and an unusually low methionine content. The protein appears to contain two, rather than the usual one, trimethyllysines. Comparative tryptic peptide mapping of scallop-gill and bovine-brain calmodulins indicates coincidence of over 75% of the major peptides, but at least two major peptides in each show no near-equivalency. Preliminary results using ATP-reactivated, permeabilized gill cell models show no effect of calcium on ciliary beat or directionality of the lateral cilia, which constitute the vast majority of the isolated cilia. Thus, the biological function of this calmodulin is unclear. Scallop-gill ciliary calmodulin may be involved in regulating dynein-tubule or spoke-tubule interaction, or it may serve some coupled calcium-transport function.

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In vitro *assembly of macrotubules and microtubules from eggs of marine invertebrates.*

Kathy A. Suprenant and Lionel I. Rebhun. University of Virginia, Charlottesville, VA.

Tubulin was purified from unfertilized and KCL-activated surf-clam eggs *(Spisula solidissima)* by DEAE-Sephadex column chromatography (Kuriyama 1977, *J. Biochem.* **81:** 1115–1125) followed by cycles of temperature-dependent assembly and disassembly. The first polymerization of *Spisula* egg extracts at 37° yields bright aggregates of linear polymers observed by dark-field light microscopy. In the electron microscope, these linear polymers appear as macrotubules 50–60 nm in diameter. The macrotubules are composed of alpha and beta tubulins as well as 10–20 other proteins ranging in molecular weight from 20,000 to 100,000. Macrotubules can be assembled from tubulin obtained from both oocytes and from isolated meiotic spindles. Electron microscopy after negative staining with uranyl acetate shows that macrotubules are formed from coiled sheets of 26–27 protofilaments. When further purified either by phosphocellulose chromatography or by several cycles of assembly and disassembly after DEAE chromatography, tubulin assembles into the more familiar 25-nm microtubules. Tubulin found in macrotubules and microtubules has identical isoelectric points of 5.8 for alpha and 5.6 for beta subunits. These results suggest that macrotubule formation results from interaction of tubulin with a non-tubulin component, rather than from an intrinsic property of tubulin. Brain MAPs, purified from cycle-purified chick brain tubulin, co-assembles stoichiometrically with *Spisula* purified alpha and beta tubulin, lowering the critical concentration for assembly at 22° from 0.4 mg/ml to 0.2 mg/ml. Brain MAPs, added to highly purified *Spisula* tubulin that would ordinarily assemble into microtubules, assemble into large coiled sheets resembling macrotubules. Macrotubules (68 nm) were also obtained by polymerizing a tubulin-containing fraction obtained by DEAE-chromatography from extracts of activated starfish oocytes. However, only microtubules were obtained after one cycle of assembly following DEAE-chromatography of extracts of unfertilized sand-dollar eggs, similar to results previously reported for sea urchin egg extracts. Sand-dollar and sea-urchin eggs are obtained in post-meiotic stages and contain a large pool of tubulin destined for assembly of mitotic microtubules. Starfish and *Spisula* eggs are obtained in meiotic stages, their tubulin destined for assembly of meiotic spindles. Our results suggest that assembly of macrotubules results from association of tubulin with a factor(s) present in meiotic oocytes but not in oocytes obtained in post-meiotic stages.

Does cytochalasin-D induce reversible disruption of the cleavage contractile ring? YUICHIRO TANAKA AND SHINYA INOUÉ (MBL, Woods Hole, MA).

The red pigment granules in fertilized *Arbacia* eggs are embedded in the cell cortex tightly enough not to be displaced by centrifugal forces that stratify the cytoplasm. These pigment granules are, therefore, suitable markers for observing the movement of the cell cortex. Cleaving sea urchin eggs are divided in two by contraction of a contractile ring. At cleavage, the pigment granules move to the furrow region, and a pigment granule band is formed in the area corresponding to the contractile ring. The contractile ring is reported to be composed of actin filaments. Cytochalasin-D specifically affects the integrity of structures containing actin filaments.

In these experiments, 5–10 μM cytochalasin-D was applied to eggs of *Arbacia punctulata*, and the behavior of the pigment granule band was observed. When 5–10 μM cytochalasin-D was applied to the furrowing egg, the pigment granule band yielded, and the furrow regressed partially. The yielding was manifested by a clear area that was of constant width and that lacked the pigment granules in the band area. The band then reformed as the cell continued to furrow. This process was repeated several times during cleavage while the egg was in cytochalasin-D sea-water. The yielding and restoration of the pigment granule band were observed during cytochalasin-D application and in eggs pretreated with 5–10 μM cytochalasin-D for 10–30 min some time before cleavage.

These results suggest that: (1) in cytochalasin-D, the substance of the contractile ring, probably actin filaments, can not support the tension of contraction and yields or is extended and (2) the contractile ring is a dynamic structure that is continuously reforming in spite of the disruption by cytochalasin-D.

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COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

Secretory tissue for aqueous humor in the eyes of shark and skate. D. EUGENE COPELAND (Marine Biological Laboratory).

The eyes of fish (both teleost and elasmobranch) are unique in that they have no ciliary muscle to move the lens for accommodation. Instead, the lens is suspended by a dorsal ligament and swung on that "hinge" by a ventrally located column of muscle (campanula of Halleri). There is no ciliary projection and pars plica comparable to the structure in mammalian eye that produces aqueous humor. Therefore, tissue between the retina and contractile iris of shark and skate was studied by electron microscopy. A two layered epithelium is found; an inner (vitreal) nonpigmented layer (NPL), subtended by a pigmented layer (PL). The NPL has numerous one-to-one, basal interdigitations and the apical ends of the cells are sealed with zonula occludentes junctors. There are no junctors between the interdigitating extensions. The NPL and PL are joined by numerous gap junctions, suggesting functional relationship. The PL has large intercellular spaces partially filled with interdigitating projections. The basal portions of the PL cells are packed with mitochondria and have a larger total population than does the NPL cells. The arrangement of the two layers suggests that both NPL and PL are involved in the active secretion of aqueous humor.

Photosynthetic response of Zostera marina *(eelgrass) to* in situ *manipulations of light.* W. C. DENNISON, D. MAUZERALL, AND R. S. ALBERTE (University of Chicago).

Zostera marina L. (eelgrass) beds constitute some of the most productive communities in the world, yet little is known about the nature or degree of adaptation of these highly specialized flowering plants to the coastal marine environment. To test for photosynthetic response, sun reflectors and light shading screens were placed in shallow (1.5 m) and deep (5.5 m) areas of an eelgrass bed to increase (+40%)

and decrease (−45%) ambient light intensities. Light intensities in shallow water at the base and tip of the leaf canopy were 80 and 1440 $\mu E \cdot m^{-2} \cdot sec^{-1}$, respectively, while they were 115 and 455 in deep water. The acclimation period of 7 days was chosen to approximate leaf turnover times (9–12 days). The study site was adjacent to the Fisheries Jetty in Great Harbor, Woods Hole, MA (August, 1981).

Light-saturated photosynthetic rates, measured by oxygen evolution, were unaffected by altered light intensities in the shallow area. However, a decrease in photosynthesis per leaf area (0.76 compared with 0.67 $\mu Mol\ O_2 \cdot dm^{-2} \cdot min^{-1}$) and per chlorophyll (0.22 compared with 0.16 $\mu Mol\ O_2 \cdot chl^{-1} \cdot min^{-1}$) was observed in the shaded plants at the deep station. No effect was observed in the increased light experiment. Leaf area population rate at the shallow site was 54 $dm^2 \cdot m^{-2} \cdot day^{-1}$ in all treatments. Leaf area production at the deep site (40 $dm^2 \cdot m^{-2} \cdot day^{-1}$) was unaffected by increased light levels, but dramatically reduced (24 $dm^2 \cdot m^{-2} \cdot day^{-1}$), by shading. There was no significant changes in leaf chlorophyll content, chlorophyll a/b ratio, $PSUO_2$ size or PSU density in any of the light manipulations.

These results indicate (1) that *Z. marina* does not adjust to light conditions by changing photosynethetic characteristics and (2) that adjustment to changing light conditions is largely by leaf area production rates. In combination with previous data, we infer that much of the daily and seasonal growth of eelgrass occurs in above saturating light levels.

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Non-invasive, long-term recording of shipworm boring activity. SCOTT M. GALLAGER (Woods Hole Oceanographic Institution), ROBERT B. BARLOW JR., AND ROGER MANN.

To study the bioenergetics of adult shipworms, we developed a simple, non-invasive technique for monitoring boring or shell rasping activity in *Teredo navalis, Bankia gouldi,* and *Lyrodus pedicellatus.* With the aid of X-radiographs of single, living shipworms in dowels, stainless steel pin electrodes were inserted into the wood near the rasping shell without contacting the animal. The electrodes were connected to a differential recording amplifier (gain 1000).

The shipworm generates action potentials that are readily recorded with this technique. Their frequency is 0.2–0.5 sec^{-1}, duration is 50 msec, and amplitude is generally in the range of 1–5 mV. The action potentials correlate with individual shell rasps as observed through a plexiglass window in the wood dowel. Thus, they may represent the electromyogram of the adductor muscle responsible for rasping. Depending on the number and placement of the electrodes, shell rotation could be displayed as changes in the potentials amplitude. The animals rotated through 360° and back again during 20–40 rasps.

Boring activity of single shipworms was compared to respiration (measured by a polarographic oxygen electrode), phytoplankton filtration rate (measured by a fluorometer) and fecal pellet production (measured by an in-line phototransistor) by placing the dowels in a flow-through chamber. Boring activity continued at a rate of about 2300 rasps/h for more than 72 h before slowing or ceasing for brief periods. Respiration was tightly coupled with boring activity and varied from 0.6 to 2.8 ml O_2/g dry weight/h in quiescent and actively rasping animals, respectively. Filtration rate was largely independent of both boring and respiratory activity. Fecal pellets were expelled continuously during boring and up to 1 h after boring had ceased.

Simultaneous rasping, respiration and filtration activity is clearly evident in undisturbed shipworms. The present recording system should prove useful in future physiological and behavioral studies.

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Sulfhydryl group catalysis of the reaction of methylglyoxal with proteins. P. R. C. GASCOYNE, J. A. MCLAUGHLIN, R. PETHIG, AND A. SZENT-GYÖRGYI.

Szent-Györgyi has drawn attention (1979, *Biol. Bull.,* **157:** 398) to the role of aldehydes such as methylglyoxal (MG) in forming charge-transfer reactions with protein molecules (Pethig and McLaughlin, 1979, *Biol. Bull.,* **157:** 388; Gascoyne, 1979, **157:** 369). Earlier experiments indicated that the reaction of MG with proteins to form pale brown products with an associated free radical content depended upon the ε-amino groups of lysine residues. This reaction is characterized by an absorbance peak at 330 nm, extending into the blue end of the visible spectrum. We added, to 6 *M* guanidine HCl solutions of bovine serum albumin, various quantities of dithiothreitol, to provide protein samples with differing free SH contents. After dialysis, the samples were reacted with 0.2 *M* MG. A correlation was found between their absorbance at 330 nm and the free SH content, as determined using Ellman's reagent. Untreated lysozyme, which contained no free SH groups, gave a very weak absorbance at 330 nm when reacted with MG. However, when SH-bearing compounds such as glutathione, thiolactic acid, or ethane dithiol accompanied the methylglyoxal treatment, absorbance was strong. Of these three thiol compounds, glutathione with its free amino group was the only one that gave rise to the 330 nm absorbance in reactions with lysine-blocked albumin. Attempts to study the effect of blocking the free SH group of albumin have so far been complicated by other reactions that confuse the absorbance spectra around 330 nm. Assays using Ellman's reagent showed that little if any free SH was consumed in the protein-methylglyoxal reactions. We conclude that the sulfhydryl groups acted as catalysts in the color reactions between MG and the lysine residues of albumin and lysozyme.

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Flatfish vestibulo-ocular reflex (VOR): Physiological and anatomical aspects.

W. Graf (Dept. Physiology and Biophysics, NYU Medical Center, 550 First Ave., New York, NY 10016).

Flatfish execute swimming movements in a similar way as upright fish, and both employ the vestibulo-oscular reflex (VOR) to stabilize the animal's visual image. Yet, in the adult flatfish, labyrinths and eye axes are oriented perpendicular to each other, and both eyes are on one side of the body, where they perform compensatory movements in the same direction. Since these conjugate eye movements are different from those observed in upright fish during a given body movement, changes in either the extraocular arrangement and/or the central nervous connectivity are required.

In *Pseudopleuronectes americanus* the sizes of the horizontal extraocular muscles (lateral and medial rectus) were smaller than the vertical eye muscles. However, the kinematic characteristics of all six extraocular muscles were not much different from these in comparable lateral-eyed animals. HRP injections into single eye muscles revealed the commonly described distribution of motoneurons in the extraocular nuclei. These motoneuron pools consisted of two contralateral (superior rectus and superior oblique), and four ipsilateral populations (inferior oblique, inferior rectus, medial rectus and lateral rectus). The labeled cells composed distinct motoneuron-pools with little overlap. As expected, the numbers of labeled motoneurons differed in horizontal and vertical eye movers. The numerical difference was especially prominent in comparing the abducens nucleus to one of the vertical recti subdivisions. Secondary vestibular axons in the medial longitudinal fasciculus were identified by stimulating single ampullary branches of the semicircular canals, and were intracellularly injected with HRP. When reconstructed, terminations were found in the different subgroups of the oculomotor complex, including adjacent and rostral areas. At first glance, the individual morphology of these neurons appears qualitatively similar to that described in specific target neurons and nuclei in mammals. Although similar experiments in upright swimming fish are needed, it appears that the central connectivity may be constant and peripheral alterations may account for the differences necessary for a functioning VOR.

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Morphology of the ocular fundi of selected marine animals in vivo.

Donald A. Grover, M.D., and Seymour Zigman, Ph.D. (University of Rochester School of Medicine and Dentistry, Rochester, NY 14642).

Our purpose is to report a procedure using readily available ophthalmoscope equipment to examine, record, and photograph the fundus of a variety of marine animals. The dogfish, skate, toadfish, sea robin, scup, and sea bass were examined by direct and indirect ophthalmoscopy. The ease and difficulty of fundoscopy and photography were recorded, as were descriptions of the intraocular morphology. Eyes of dogfish and toadfish were the easiest to examine, but acceptable photography and examinations were accomplished on the sea bass, scup, and sea robin. The squid eye was easily examined but was photographed in only a limited fashion. A very limited examination was done on the skate, but no photography was accomplished. The value of such intraocular fundoscopy and photography is that it permits serial examination and documentation of time-related changes in the living eye. This would permit rapid screening of the ocular fundi of several marine animals, and allow quick selection of eyes for histopathological analysis. An example of the use of this procedure is the comparison of the dark-adapted and light-adapted states of the toadfish eye. The light-adapted eye shows an orange fundus with mottled, pigmented areas and a cross-hatched screening at the level of the retina. In contrast, the dark-adapted eye shows multiple grayish-white striae radiating out from the optic nerve. These striae are so opaque that neither the underlying orange fundus nor its pigment mottling can clearly be discerned. Upon return to the light-adapted state, the orange fundus and pigment can again be seen.

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Carbohydrate transport by Vibrio harveyi. HANS KORNBERG (Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U. K.) AND JUDY KUDLACZ.

The marine luminescent bacterium *Vibrio harveyi* resembles *Escherichia coli* in possessing a phosphoenolpyruvate-dependent phosphotransferase (PT) system that catalyzes the uptake of glucose and fructose concomitant with their phosphorylation to the 6- and 1-phosphate esters respectively. In both organisms, glucose inhibits fructose uptake. An unexpected difference between these enteric bacteria became apparent from the effects of non-catabolizable analogs of glucose (methyl α-glucoside, 2-deoxyglucose, 5-thioglucose) on growth and fructose uptake. In *E. coli*, these analogs strongly inhibit the uptake of all sugars transported via the PT system; in *V. harveyi*, the inhibition both of the uptake of, and growth on, PT-sugars is partial, and the inhibition of growth is overcome in about one doubling. These findings— (i) that glucose analogs only inhibit partially and (ii) that cells readily escape growth inhibition by these analogs—are explained by:

(i) the presence in *V. harveyi* of active transport system(s) that also effect the uptake of fructose and glucose but not of, *e.g.*, methyl α-glucoside. These systems are powerfully inhibited by 5 μM-carbonyl cyanide m-chlorophenylhydrazone (CCCP) and are absent from *E. coli*; fructose uptake by *V. harveyi* is also abolished by 10 mM-arsenate;

(ii) the loss of PT-activity, measured as loss of the ability to take up methyl α-glucoside, as *V. harveyi* grow on fructose or mannitol in the presence of non-catabolizable glucose analogs. In cells that have escaped from the inhibition of growth by these analogs, glucose and the analogs have largely lost their inhibitory effects on fructose uptake. In such cells, further growth on PT-sugars therefore probably proceeds by mechanisms that complement and may replace the PT-system that is known to predominate in terrestrial enteric bacteria, such as *E. coli* and the related marine *Vibrio parahaemolyticus.*

Quantitative determination of protein synthesis in toadfish by phenylalanine swamping. RITA W. MATHEWS AND AUDREY E. V. HASCHEMEYER (Hunter College)

This study explores the use of a new method, originally developed in rat, for measuring protein synthetic rates in tissues of fish. Male toadfish, *Opsanus tau*, 230 ± 30 g body weight, were lightly anesthetized with benzocaine (0.2 g/l) and maintained with well aerated circulating seawater during hepatic portal vein injection of ^{3}H-phenylalanine (0.34 μCi/μmole) at a dose of 1.4 μmole/g body wt. After a free-swimming period of 20 min to 3 h at 22°C, or 3 h to 15 h at 11°C, the fish was killed, and liver and white epaxial muscle were collected in liquid nitrogen. Protein and free-amino-acid fractions were prepared from the frozen tissues and analyzed for radioactivity by scintillation counting and for L-phenylalanine by fluorescence after conversion to β-phenethylamine.

The results showed an initial rapid uptake of phenylalanine by toadfish liver (to 2.5 μmole/g), declining thereafter. Levels in muscle rose more slowly and remained constant (1.6 μmole/g) for 1 to 3 h at 22°C. Specific radioactivity of free phenylalanine (S_A) peaked at 1 h (22°C) or 5 h (11°C). A subsequent decline apparently was due to ^{3}H exchange. Protein synthetic rate k_s (percentage of tissue protein synthesized per day) was determined from the difference in specific radioactivity of protein-bound phenylalanine at 20 min and 3 h at 22°C (or 5 and 15 h at 11°C) divided by the average S_A for the interval. Values of k_s for groups of 3–5 fish averaged 14% and 2.5% in liver and 0.60% and 0.15% in muscle at 22°C and 11°C, respectively. The results in liver agree well with those previously calculated from elongation rates and ribosome concentration.

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Competition properties of leucine transport in liver of cold-acclimated toadfish. ROGER PERSELL AND AUDREY E. V. HASCHEMEYER (Hunter College).

Previous studies have shown that L-leucine transport by toadfish liver *in vivo* occurs by a saturable carrier-mediated process, as in other animal cells. In summer fish at 22°C, 40% of uptake occurs by active transport; major competitors for the pathway are isoleucine and phenylalanine. When fish are cooled to 10°, the active transport component of uptake disappears, saturation parameters change, and other amino acids, particularly methionine and histidine, become significant competitors. The present study examines the effect of cold acclimation on the properties of this system.

Male toadfish, *Opsanus tau* (288 ± 31 g body weight) were acclimated at 10° for 1–21 days. Fish were injected through the hepatic portal vein with 0.1 ml of 0.1 mM ^{14}C-leucine, tracer ^{3}H-inulin, and competing amino acids at 15 m*M*. The distribution of the two isotopes in liver and in blood leaving the hepatic vein was analyzed to determine intracellular uptake of ^{14}C-leucine. Fish kept 1 day at 10° before assay at 10° showed high levels of inhibition of leucine uptake by methionine (65%) and histidine (42%), comparable to values previously obtained immediately after cooling. Inhibition increased slightly at 4 days, followed by a rapid decline at 10 and 14 days toward levels observed in 20°-acclimated fish at 20°. Saturation behavior, tested with 15 m*M* leucine, also showed compensation with 14-day acclimation. These results provide the first evidence of recovery of a cold-sensitive amino acid transport system through cold acclimation.

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Net flux of dissolved amino acids in adults and larvae of the shipworm Lyrodus pedicellatus *Quatrefages.* ROBERT D. PRUSCH, SCOTT M. GALLAGER, AND ROGER MANN.

The influx and net flux of alanine in the adult and pediveliger larvae of the shipworm *Lyrodus pedicellatus* were examined. Either individual adults in wooden dowels or *ca.* 1000 larvae were incubated for up to 3 h in 10–15 ml of filtered sea water containing 2–150 μM alanine. The time course of depletion of alanine in the medium was documented using both ^{14}C-labeled alanine (influx) and the fluorescamine technique (net flux).

Influx was comparable to net flux at ambient concentrations greater than 40 μM but somewhat higher than net flux at concentrations of 40 μM and less for both adults and larvae. Saturable maximum uptake rates were an order of magnitude greater for larvae than adults (0.35 and 0.035 μM/mg dry wt/h, respectively). Inhibition of influx of 20 μM alanine exceeded 50% for both adults and larvae in the presence of 20 μM glycine suggesting that transport of alanine in *L. pedicellatus* is carrier-mediated. Experiments with adults excised from the wood and with ligatured siphons indicate that influx via the external mantle epithelium is minimal.

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A complete transcription and translation system for Escherichia coli: *Improvement of DNA-dependent protein synthesis by nuclease treatment and the addition of polyamines.* BETH A. RASMUSSEN (Univ. of North Carolina, Dept. of Bact. and Immunol.) and TIM HUNT.

In 1970, Zubay published a procedure for making a cell-free protein synthesis system using an extract of the bacterium *E. coli* (Zubay, G. (1970) *The Lactose Operon*, Cold Spring Harbor, p. 375). We wished to improve the system, which we found to have a high background incorporation further stimulated by the addition of any DNA. We removed the background by treating the system with micrococcal nuclease and substituting spermidine for the Ca^{++} necessary in the classical Zubay system. We found this substitution improved the system by 2–3 fold. The system was set up as described in *Experiments in Molecular Genetics* (Miller, J. H. (1972) Cold Spring Harbor, p. 419) with the following changes: (1) The S30 extract was treated with nuclease; 100 μl of S30 extract was combined with 1 μl 0.1 *M* $CaCl_2$ and 2 μl micrococcal nuclease (1 mg/ml, 15,000 U/mg) and incubated for 20 min at 15°C. The nuclease was then inactivated by the addition of 1 μl 0.2 *M* K-EGTA, pH 7.0. (2) Potassium acetate, magnesium acetate, calcium chloride, amino acids, and water were omitted from the "reaction mixture." (3) The incubation mix was composed of 16 μl "reaction mixture," 10 μl PEG 6000 (160 mg/ml), 5 μl 20× amino acids without methionine, 10 μl spermidine-spermine mixture (18 m*M* spermidine, 2 m*M* spermine), 5 μl potassium acetate-magnesium acetate (0.71 *M* potassium acetate, 0.14 *M* magnesium acetate), 10 μl water, 2 μl 3'5' cAMP (50 m*M*), 3 μl ^{35}S methionine (10 mCi/ml), 10 μl DNA (1–2 mg/ml), and 30 μl nuclease treated S30 extract.

Nuclease treatment of the S30 extract made the system highly dependent upon the addition of DNA (and the synthesis of RNA) for the production of protein. Using the ColE1 plasmid DNA, we obtained synthesis of active colicin E1, which could be detected at a dilution of over 600-fold of the incubation mixture. Comparison of the protein products produced using the ColE1 plasmid in the nuclease-treated mix with that produced in a Maxi-Cell system revealed a prominent protein of just under 56,000 d made in both systems, and several smaller proteins made in the *in vitro* system. From this we conclude that nuclease treatment of the S30 extract can be used to make DNA-dependent protein synthesis system capable of accurate transcription and translation.

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Reproductive effort of molluscs in bioenergetic terms: some computational methods. W. D. RUSSELL-HUNTER AND FRANK A. ROMANO, III (Syracuse University).

Much current evolutionary discussion concerns differences of reproductive allocation in closely-related forms. Lacking direct energy-flux data, ratios of reproductive effort (R.E.) are computed in terms of organic carbon from productivity rates. A survey of molluscan data (Browne and Russell-Hunter 1978, *Oecologia (Berlin)* **37**: 23–27.), employed two indices: the percentage of annualized non-respired assimilation (N-R.A.) channeled into reproduction, and the ratio between the carbon channeled into reproduction and the carbon contained in the average female (or adult hermaphrodite) at the time of reproduction.

An inherent weakness in the much-used second ratio is that it involves a rate output for reproduction and a biomass estimate for the parent. Fourfold within-species differences in percentage N-R.A., and approximately threefold differences in the second R.E. ratio, are found in *Laevapex* and *Ferrissia* (between populations, and between generations in bivoltine stocks). Both estimates of R.E. are based on modal adult biomass values without reference to differences in immediate bioenergetic history (in particular to growth *rates* achieved immediately before reproduction). For *Melampus* (Russell-Hunter *et al.* 1972, *Biol. Bull.* 143: 623–656.), R.E. was 46% of annualized N-R.A., 87% of breeding-season N-R.A., or 32% if spring predbreeding N-R.A. had been sustained.

From suitable data, a dynamic index of R.E. could be reproductive output *rate* divided by a productivity *rate* computed for the immediate period of prebreeding growth. For the two generations of (bivoltine) *Ferrissia*, this computes as 0.802 and 0.886 (compared with 73% and 37% N-R.A.). Infraspecific differences are largely cancelled out, and this index may be closer to a genetically determined rate-function for potential R.E., which is then modified by the biological state of each cohort and by the season. Eventually it may become possible to incorporate parallel data on catabolic partitioning (as related to different levels of anabolic accretion) in more complete actuarial bioenergetics of R.E.

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Optical recordings of action potential propagation in intact heart. GUY SALAMA (University of Pittsburgh, School of Medicine, Pittsburgh PA 15261), T. SANGER, L. B. COHEN.

Voltage-sensitive dyes (WW375 750 ± 30 nm and NK2367 720 ± 30 nm) known to linearly follow transmembrane potential changes in heart were used to simultaneously monitor action potentials from multiple regions of a whole heart. Frog hearts (*Rana catesbiana*) were excised, perfused by venous or cordis bulbus canulation, and illuminated by two 100-W tungsten halogen lamps, stained in 0.4 to 1.2 mg/ml of dye in Ringer's solution for 10–20 min, then washed of excess dye in zero Ca^{+2} Ringers (used to subdue contractions). An image of the whole heart or a selected segment of tissue was focused on a 12 × 12 element photodiode array by a lens providing variable magnification and aperture. The aperture was reduced to increase the depth-of-field and the heart was rested against a glass plate to reduce its natural curvature. Analogue outputs from 124 elements of the array were amplified, AC coupled using a 3-sec time constant to preserve the shape of the action potential, and then fed to a PDP-11/34 minicomputer. Signals from each (1.4 × 1.4 mm) element of the array were sampled every 0.8 msec, and stored for subsequent analysis. A series of linear regressions was performed on each optical trace to determine the time of the maximum rate of rise, which was taken to be the time at which the most cells observed by that element were activated. The algorithm was capable of locating action potentials superimposed on motion artifacts 20 times greater in amplitude. Differences in electrical activity recorded by neighboring elements allowed the mapping of propagation pathways. Delays in the firing of action potentials between neighboring 1.4 mm regions of tissue varied from 4–6 msec, and between atrium and ventricle varied from 300–500 msec. The spatial (1.4 mm) and temporal (0.8 msec) resolution of the apparatus allowed us to investigate the patterns of electrical activity caused by ionic substitution or drug intervention. For instance, in high K^+ (6 to 10 m*M*), the rate of rise of the optical action potentials decreased by a factor of two with a slight decrease in conduction velocity; propranolol (2 mg/100 ml) lengthened the duration of the action potential by 1.5× and reduced the conduction velocity by 1.4×. Patterns of electrical activity determined by such a rapid non-invasive method offer a complete picture of cardiac excitation under normal and pathological conditions and can point out sites of local injury and their effect on propagation pathways.

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Membrane transport of the essential amino acid leucine in the gut of Arbacia punctulata—*effect of Hg^{++} and other heavy metals.* ROBIN SOCCI, VICTOR INNACCONE, AND A. FARMANFARMAIAN (Rutgers University, Piscataway, NJ).

Adult sea urchins are generally slow moving intertidal and subtidal benthic animals. They are omnivores but usually feed on algae. Man, marine mammals, birds, fish, and crustacea eat the roe and other soft parts of sea urchins. Developmental and molecular bioligists throughout the world make extensive use of sea urchin eggs and sperm for experimental work.

In coastal waters which receive industrial and urban sewage effluents, sea urchins are exposed to a variety of heavy metal pollutants in the water column, the sediment, and via ingestion of food contaminated by the bio-accumulation of these toxicants. Since several heavy metals inhibit enzymatic and transport activity, we examined the effect of $HgCl_2$, CH_3HgCl, $CoCl_2$, $CdCl_2$, and $NaVO_3$ on the intestinal absorbtion of 0.25 m*M* L-leucine in filtered sea water, pH 7.8 at 20°C using *Arbacia* intestinal tissue incubated 15 min *in vitro*. The rate of uptake and the terminal tissue-to-medium concentration ratios were computed from counts of ^{14}C-labeled leucine after correction for ^3H-inulin space. The difference in mean rates and ratios were considered significant at $P \leq 0.05$.

The results showed that *Arbacia* intestinal tissue is able to accumulate leucine up to 40 fold. The rate of uptake was significantly inhibited 7% by 0.5-, 17% by 1-, 21% by 2.0- and 46% by 10-ppm Hg^{++}. By contrast, CH_3Hg^+ inhibited uptake significantly only at 10 ppm (13%) and 20 ppm (26%), implying that the charges on the divalent Hg^{++} ion are essential for effective carrier inhibition. The other heavy metals tested (Cd^{++}, Co^{++}, and VO_3^-) did not inhibit uptake significantly in the range of 0.5–20 ppm.

Supported by the Center for Coastal and Environmental Studies, Rutgers University and New Jersey Sea Grant.

Amines increases the strength of Limulus *heart contractions by enhancing neuromuscular transmission.* WINSOR H. WATSON, III (Zoology Dept., University of New Hampshire) AND TOSHINORI HOSHI.

The neurogenic *Limulus* heart is nodulated by amines and the neuropeptide protolin. Octopamine, dopamine, norepinephrine, and epinephrine, as well as a proctolin-like peptide, are present in the cardiac ganglion. Previous work has shown that amines increase both the rate and strength of heart contractions, and that proctolin augments the amplitude of heart contractions by increasing myocardium contractility. The amines act on the pacemaker cells and follower cells in the cardiac ganglion to increase the heart rate. The pacemaker cells initiate the heart beat and drive the follower cells, which innervate the cardiac muscle. The purpose of this study was to determine the mechanism underlying the inotropic effects of dopamine (DA) and octopamine (OCT).

Intracellular recordings were obtained from follower cells and muscle fibers of spontaneously beating hearts while the strength of heart contractions was being monitored. Bath application of DA and OCT increased the rate and strength of heart contractions, decreased the duration of follower cell bursts, decreased the number of spikes per burst, and increased in the size of individual EJPs. There was no consistent change in the apparent input resistance or resting membrane potential of the muscle fibers.

To further examine the effects of DA and OCT on the amplitude of EJPs, we elicited single EJPs in muscle fibers by stimulating follower cell axons directly. DA(10^{-6} *M* and 10^{-5} *M*) increased EJP amplitude by 64% (SD = 45% $n = 7$) and 109% (SD = 50% $n = 5$), respectively. OCT (10^{-5} *M*) increased the amplitude by 110% (SD = 84% $n = 4$). These figures are in agreement with the percent increase in contraction strength induced in the whole heart by the same concentrations of the respective amines.

Thus, the overall effect of amines on the *Limulus* heart is to (1) increase the heart rate by acting on pacemaker cells, (2) decrease the burst duration and number of spikes in follower cells, and (3) increase contraction strength by enhancing neuromuscular transmission between the follower cells and myocardium. This is functionally analogous to the effect of amines on the vertebrate heart, although the exact mechanisms are obviously different. Future work will focus on possible synergistic interactions between the amines and proctolin.

This work was funded by the New Hampshire Heart Association, a Step Towards Independence Fellowship, and a Summer Faculty Fellowship.

DEVELOPMENTAL BIOLOGY

Near-UV light sensitizes cells to chemical damage. BLENDA ANTONELLIS AND SEYMOUR ZIGMAN (University of Rochester, Rochester, NY 14642).

Photosensitization is the process whereby chemicals enhance light-induced cellular damage. Near-UV light has now been found to sensitize the damage to cells caused by chemical agents (*i.e.*, chemosensitization). In these experiments combinations of near-UV radiation in the range 300–400 nm and retinoic acid (RA) were tested as mitotic inhibitors of sea urchin eggs (*Arbacia punctulata*). Initially all-*trans* retinol, retinal, and retinoic acid were tested as near-UV photosensitizers, but with negative results. When unfertilized eggs were pre-irradiated with near-UV light (at an irradiance of 1 mW/cm^2) for time periods of not less than 3 h, and then RA (10^{-4} *M*) was added to the sea water before or after fertilization, cell division was totally prevented. Near-UV light and RA effects on sea urchin egg DNA synthesis were studied by measuring the incorporation of ^{3}H-thymidine into TCA precipitates counted on filter paper pads using Econofluor liquid scintillation counting. RA alone (at 10^{-4} *M*) depressed the incorporation of thymidine into DNA appreciably, compared with controls or near-UV exposed eggs. Eggs exposed to near-UV light for 3 h and then to RA as above, totally stopped incorporation of ^{3}H-thymidine into DNA. Cell division was also depressed by 99% by near-UV followed by RA exposure. Such effects were not observed in similar experiments using retinol or retinal to near-UV exposed eggs. These findings support the concept that near-UV light can serve as a chemosensitizer specifically for the RA-mediated prevention of cell division and inhibition of DNA synthesis in sea urchin eggs.

Support: N.E.I. and Research to Prevent Blindness, Inc. Grants.

Experimental evidence for the origins of mosaic development in the polyclad Turbellaria. BARBARA C. BOYER (Union College).

Cell deletion experiments were carried out on the embryo of the polyclad turbellarian *Hoploplana inquilina* to further examine the nature of development in primitive spiralians. The polyclads are of particular interest because they provide a link between the acoels with regulative development (Boyer 1971, *J. Exp. Zool.* **176:** 96–105) and the determinative annelids and molluscs.

Single blastomeres were deleted at the two- and four-cell stages by punctures through the egg membranes with tungsten needles. Fifty-one complete deletions of one cell at the two-cell stage resulted in partial larvae that were abnormal in body shape, lobe development, and swimming behavior. Since 33% of these had no eyes and 55% had only one eye, developmental potentials of the blastomeres appear to differ even at the two-cell stage. Incomplete deletions increased the proportion of one- and two-eyed larvae, suggesting an interaction between these cells in normal development to form two eyes.

Twenty-one complete deletions of one cell at the four-cell stage gave rise to larvae with a recognizable Mullers larva shape, but usually with specific regional deficiencies in the anterior-dorsal, posterior-ventral, right, or left quadrants. Eighty-one percent had one eye and 19% had two eyes; these proportions suggest that deletion of any of three blastomeres results in loss of one eye. This is consistent with the hypothesis that two of the cells are in the direct eye lineage but the presence of a third is necessary for the development of two eyes.

This study demonstrates that the embryo of *Hoploplana* is determined early, as half and three-quarter embryos form deficient larvae, and shows that cell interactions play an important role in development. It also provides evidence that mosaicism became associated with spiral cleavage in the quartet form during the evolution of the Turbellaria.

This work was supported by an MBL Steps Toward Independence Fellowship.

A comparison between the activity of ribosomes from eggs and early embryos of Arbacia punctulata. TABBY DONIACH AND TIM HUNT (MRC Laboratory of Molecular Biology, Cambridge, England).

Fertilization of sea urchin eggs triggers a dramatic increase in the rate of protein synthesis. We compared the activity of ribosomes from eggs and early embryos in a ribosome-dependent system containing rabbit reticulocyte S100 and crude initiation factors. Sea urchin extracts were prepared by homogenizing *Arbacia* eggs or early embryos in one volume of buffer (Winkler and Steinhardt, 1981, *Dev. Biol.* **84:** 432–439) at pH 7.4, centrifuging them 20 min at 12,000 × *g*, freezing them in liquid N_2, and storing them at −70°C. When the extracts were thawed, 5 min centrifugation in a microfuge removed an inhibitor of initiation of protein synthesis. The resulting supernatant, containing ribosomes, was used for subsequent experiments. Protein synthesis in the hybrid rabbit-urchin system was dependent on both the initiation factors and the S100, and was strongly stimulated by exogenous mRNA. We noticed that the urchin ribosomes are essentially all in the form of 80S couples, with almost no 60S and 40S subunits; adding the initiation factors and S100 dissociated up to 30% of the 80S monomers from eggs or embryos into subunits. The rate of travel of ribosomes along tobacco mosaic virus RNA was measured by timing the appearance of the 110,000 M_r translation product of this mRNA; it took about 50 min to appear (at 20°), whether egg, embryo, or rabbit ribosomes were present. The capacity of the ribosomes to form 40S and 80S initiation complexes was measured in this system using ^{35}S Met-$tRNA_f$ added in the presence of emetine, with TMV RNA as message. No significant differences between egg and embryo ribosomes emerged. Thus, by the criteria we have used, there is no difference in the properties of ribosomes before and after fertilization; but we hesitate to conclude that such differences do not exist *in vivo*.

This work was supported by NSPSH training grant 07784.

Detection of sequences homologous to a Lytechinus pictus *β-tubulin cDNA probe in a* Drosophila melanogaster *genomic library.* JOANNA L. GRODEN (The New York Blood Center) and ELIZABETH E. BROWN.

Developmentally regulated gene expression can be studied most easily in evolutionarily conserved genes with an abundant gene product, such as the genes for α- and β-tubulin. Genetic and biochemical studies of the tubulin genes of *Drosophila melanogaster* reveal at least three distinct genes for β-tubulin. In addition to a ubiquitous β-tubulin (β_1), expressed throughout development, there is a tissue specific β-tubulin (β_2) expressed only in the testis (Kemphues *et al.*, 1979, *P.N.A.S.* **76:** 3991–3995.), and a third β-tubulin (β_3) whose expression is temporally specific to a period in early embryogenesis (Raff *et al.*, submitted). Further study of tubulin gene regulation is possible using cloned sequences from these *Drosophila* β-tubulin genes. The evolutionary conservation of tubulin sequences has allowed us to select putative *Drosophila* β-tubulin clones with a sea urchin probe.

In this study, we have demonstrated the homology between *Drosophila* genomic sequences and a plasmid containing cDNA derived from a *Lytechinus pictus* β-tubulin mRNA. By Southern analysis (1975, *J. Mol. Biol.* **98:** 503–517), we have shown that labeled *Lytechinus* β-tubulin probe will hybridize to at least two fragments of EcoRI digested *Drosophila* genomic DNA. The *Lytechinus* β-tubulin clone was then used as a probe to select homologous sequences from a *Drosophila* genomic (lambda) library (gift of T. Maniatis), using the plaque hybridization technique of Benton and Davis (1977, *Science* **196:** 180–182). An initial screen yielded eight plaques that hybridized to the probe. Four of these plaques were rescreened and again they hybridized to the probe. Further analysis of the selected recombinant phage will indicate whether they are indeed cloned *Drosophila* β-tubulin genes, as well as which tubulins they represent.

We thank D. Alexandraki and J. Ruderman for the *L. pictus* β-tubulin clone and E. C. Raff for sponsorship. We also thank A. Bruskin and D. Wells for technical instruction.

A yellow crescent cytoskeleton in Styela *embryos.* WILLIAM R. JEFFERY (University of Texas) AND JUDITH V. HENDERSON.

Classical studies have shown that the pigmented regions of *Styela* eggs mark the boundaries of cytoplasmic areas with specific morphogenetic fates. To determine the relationship between these cytoplasmic localizations and the cytoskeleton, eggs and embryos were extracted with 0.5% Triton X-100 in 100 m*M* KCl, 2.5 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 300 m*M* Sucrose, and 10 m*M* HEPES (pH 6.9). The detergent-insoluble residues were examined by biochemical methods and by scanning (SEM) and transmission (TEM) microscopy. The cytoskeletons of eggs and embryos contain 22% of the total protein, 29% of the total lipid, 25% of the total RNA, and 75% of the total poly(A). SEM analysis showed that a meshwork of smooth fibers was preserved above the yellow crescent region of the cytoskeleton. This meshwork probably represents a lamina of membrane proteins closely associated with an underlying filamentous cytoskeleton. The lamina is present as individual, small patches in the cortex of the unfertilized egg directly above the yellow pigment granules. After fertilization, the patches form a cap above the yellow crescent and are specifically segregated during embryogenesis to the blastomeres of the mesenchyme and muscle lineages. TEM analysis showed that the yellow crescent region and its derivative blastomeres contain an elaborate cytoskeleton composed of filaments ranging from 100–150 Å in diameter. The yellow pigment granules appear to be attached to these filaments. The muscle and mesenchyme cells of the 32-cell embryo also stain positively with a monoclonal mouse antivimentin, indicating that this intermediate filament protein may be enriched in these cells. The cells of the ectodermal and endodermal lineages appear to have cytoskeletons organized differently from those inheriting the yellow crescent. The results suggest that the cytoplasmic localizations of *Styela* eggs, particularly the yellow crescent region, are underlaid by distinct cytoskeletal organizations. These cytoskeletal organizations may be related to the processes of cytoplasmic determination that occur in these regions.

Lilian Vaughan Morgan 1870–1952. KATHERINE KEENAN (Yeshiva University). Dept. of Biology, 500 W. 185th St., New York, N. Y. 10033).

Lilian Morgan was an important geneticist whose work was obscured by the attention given her husband, T. H. Morgan. Her diverse career as a biologist touched two centuries. Her work in the 1890s was mostly in descriptive zoology, while from the early- to mid-twentieth century she studied experimental genetics.

Probably her most significant scientific contribution was the discovery of the attached-X chromosome in *Drosophila* in 1921. This discovery provided further confirmation of the chromosome theory, became a major tool for X chromosome mutational analysis, and was an important system used to demonstrate crossing over at the four-strand stage of meiosis.

She was born Lilian Vaughan Sampson and grew up in Philadelphia. She received both A.B. and M.A. degrees from Bryn Mawr college, where her advisor was M. Carey Thomas and where she studied zoology with E. B. Wilson. After graduation she spent a year studying chitons with the comparative anatomist Arnold Lang at the University of Zurich. She returned to spend many summers at the Marine Biological Laboratory as an independent investigator and to do research in T. H. Morgan's laboratory at Bryn Mawr. From 1895 to 1906 she published papers on the musculature of chitons, breeding and development in anurans, and development and regeneration in flatworms.

In 1904, Lilian Sampson and T. H. Morgan were married. They raised four children in a happy, active family. Lilian did not publish again until the children were grown. In T. H. Morgan's laboratory at both Columbia University and the California Institute of Technology she had her own laboratory area and always worked independently on her own research projects. She is listed in the 1949 edition of "American Men in Science" and in her lifetime published 16 single author papers.

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Absence of regional differences in the membrane properties from the embryo of the mud snail Ilyanassa obsoleta. MARC MOREAU AND PIERRE GUERRIER (Station Biologique, 29211 Roscoff, France).

Regional differences in membrane properties related to the polar axis have been shown to exist in the egg of the scaphopod *Dentalium* (Jaffe and Guerrier 1981, *Dev. Biol.* **83:** 370–373). Thus polar lobes isolated from trefoil stage embryos were found to be more excitable than the corresponding animal cells and gave typical action potentials. To assess the developmental significance of such a mosaic pattern, we tried to extend our study to the egg of *Ilyanassa*, in which the polar lobe has the same influence as in *Dentalium*.

Unfertilized eggs, whether collected just after laying or from capsules that contained fully developed sister snails, always showed a low K^+-ion-independent resting potential (-17 mV ± 8, $n = 18$). Typical action potential were obtained (amplitude + 94 mV ± 8; duration 5 to 90 sec), which probably reflect an increase in Ca^{2+} permeability, since they are suppressed by Co^{2+} and D600. The input resistance near the resting potential was 37–60 megohms.

Membrane potential of fertilized eggs stayed essentially constant from 1st pb (polar body) to polar lobe formation. The following values were collected: at 1st pb -75 mV ± 4, $n = 4$; at pear shaped stage for 2nd pb, -76 mV ± 6, $n = 8$; at 2nd pb, -74 mV ± 4, $n = 21$; at pear shaped stage for first cleavage -75 mV ± 4, $n = 14$. These values mainly depend upon a selective permeability to K^+ ions: At any stage, a 55 mV depolarization was obtained, following a tenfold change in the external K^+ ion concentration. The Na^+ to K^+ permeability ratio was 0.019 ± 0.003, $n = 8$, and remained constant from first-pb to polar-lobe formation. The fertilized eggs appear unexcitable: no action potential could be evoked, even when polarizing the membrane in the range from -60 mV to $+10$ mV ($n = 11$). This inexcitability is clearly shown by the linear current-voltage relations used to calculate the values for input resistance: (in megohms), 1st pb, 25 ± 7, $n = 4$; pear shaped stage for second pb, 21 ± 3, $n = 7$; 2nd pb, 18 ± 9, $n = 15$; pear shaped for first cleavage 47 ± 24, $n = 10$. No differences were observed either at the level of the resting potential, when comparing isolated QQ cells (−72 mV ± 3, $n = 8$), polar lobes (−72 mV ± 3, $n = 11$) or intact trefoils (−72 ± 3, $n = 4$) or at the level of the K^+ selectivity or Na^+ to K^+ permeability ratio of the membrane. Isolated parts proved to be inexcitable, even after depolarizing polar lobes ($n = 8$), whose input calculated resistances were 22 ± 10 megohms, $n = 12$. It thus appears that our observations on *Dentalium* cannot be generalized as to the significance of membrane excitability in relation to mosaic development.

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Mitochondrial origin of an extremely prevalent set of transcripts in sea urchin embryos. David A. O'Brochta, John Speith, Rudolf Raff, Bruce P. Brandhorst, William H. Klein, and Dan Wells (Marine Biological Laboratory).

Eggs and embryos of *Strongylocentrotus purpuratus* contain a set of transcripts which bind to oligo(dT)-cellulose and are present at about 2,000,000 copies per egg. To determine if these transcripts code for protein, we selected them from gastrula RNA by hybridization to a homologous cDNA clone (#9) using four levels of stringency. The selected RNA was translated in a rabbit reticulocyte lysate and the products were analyzed by electrophoresis. No translation products were detected, though an actin cDNA clone selected translatable RNA in a parallel experiment. Nick-translated #9 DNA, mitochondrial DNA, and sperm DNA were hybridized to blots of total gastrula RNA. Mitochondrial and #9 DNA hybridized to an identical set of six transcripts ranging in size from 560–1500 nt; sperm DNA did not hybridize detectibly. Nick-translated #9 DNA was hybridized to blots of mitochondrial DNA or sperm DNA digested with EcoR1. Intense hybridization to mitochondrial DNA and faint hybridization to sperm DNA were observed. When blots of #9 DNA, mitochondrial DNA, and actin DNA, all restricted with Hind II, were probed with ^{32}P-mitochondrial DNA, there was intense hybridization to fragments of #9 and mitochondrial DNA, but not to actin DNA, a known nuclear sequence. Thus these very prevalent transcripts are homologous to sequences in the mitochondrial genome. That they are not translated in a reticulocyte lysate suggests that they are mitochondrial transcripts.

Characterization of histone mRNPs isolated from sea urchin eggs. J. T. O'Loughlin and P. R. Gross (Marine Biological Laboratory).

Maternal mRNA coding for histones exists in the cytoplasm of sea urchin eggs as free ribonucleoprotein particles sedimenting at 16 and 27 S. Following their centrifugation through two sucrose gradients, these particles are obtained only slightly contaminated (*ca.* 7%). The particles were methylated reductively with ^{14}C-formaldehyde and purified further by isopycnic banding in Metrizamide. In this nonionic medium, both classes of particle have a buoyant density of 1.2 g/cm^3, which is characteristic for mRNPs. Analysis of the protein components of the purified particles by electrophoresis on SDS-polyacrylamide gels reveals the presence of from nine to fifteen polypeptides, with those of MW 75,000 essentially identical for both classes. RNA from the particles was phenol-extracted, purified, and used to direct protein synthesis in a cell-free wheat germ system. The labeled proteins (^3H-lysine) were identified as histones by their migration in SDS and Triton-acetic acid-urea electrophoretic gels. The products of synthesis were the same for RNA from both kinds of particle. On a weight basis, however, RNA from the smaller (16S) particles was translated six times as efficiently as that from the larger class. Neither the wheat germ system nor a reticulocyte lysate system was able to translate the RNA of *intact* particles. Gel electrophoresis of the purified RNAs under denaturing conditions shows that there are several *non-coding* polynucleotides in each particle class. We are exploring possible mechanisms of translational activation, *in vivo* and *in vitro*, of the intact ribonucleoprotein particles.

Cryogenics and preservation of squid (Loligo pealei) *embryos.* Leon Shiman (Mass. Inst. of Tech.) and Anita Honkanen.

In experiments on fresh squid eggs, about 90% of embryos in stages 24–28 remained viable after being held at −16°C for 1 h in a 0.25 *M* glycerol/sea-water solution. These cryopreservation protocols bring a systematic experimental approach to squid development and genetics within reach for the first time.

Jelly casings were first removed from egg strands. Ten-embryo masses were placed in 10 × 75 mm test tubes. Between 0.5 and 0.8 ml freezing solution was added. Freezing solutions consisted of filtered seawater with glycerol as cryopreservative agent (CPA). Styrofoam-insulated samples were frozen in

−35°C and −16°C freezers for set times. Samples were thawed at approx. 30°C/min. Thawed samples were immediately washed and subsequently held in fresh running seawater.

Control experiments showed significant damage to embryos frozen and thawed in the absence of CPAs. Main experimental conditions were stage of embryonic development, concentration and type of CPA, and freezing rate. We found that very early stages did not survive freezing; however 61% of stage 11–12 embryos survived cooling to −30°C, while 80–100% of stage 24–28 embryos survived under the same conditions. But it was only stage 24–28 embryos which survived cooling and subsequent holding at −16°C for 1 h.

We found: (1) low concentrations of glycerol (0.25 *M*) proved most effective; (2) late stages of embryonic development (24–28) proved optimal for surviving freezing; (3) survival rates diminished rapidly for embryos held longer than 1 h at −16°C; (4) the effects of both CPA and freeze-thaw procedures correlate with distinct metabolic and morphologic phases of the embryo.

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Translational regulation in sea urchin embryos. DAN E. WELLS, RICHARD M. SHOWMAN, WILLIAM H. KLEIN, AND RUDOLF A. RAFF (Indiana University).

It has been shown that H3 mRNA is under sequence specific translational regulation in *Strongylocentrotus purpuratus* embryos (Wells *et al.* 1981, *Nature* **292:** 477). The purpose of this study was to show whether mRNAs for H1 and H2B in *S. purpuratus* and *Lytechinus pictus* embryos are under similar translational regulation. Polysomes were prepared from eggs and early embryos of *S. purpuratus* or *L. pictus* and fractionated on sucrose gradients. The RNA from each fraction was phenol extracted, electrophoresed in formaldehyde agarose gels, and blotted onto nitrocellulose filters. The amount of H1, H2B, and H3 mRNAs in each fraction was assayed by hybridizing labeled cloned DNA probes. In *S. purpuratus*, both H2B and H1 mRNAs remain in the inactive mRNA pool for up to 2 h after fertilization before they are actively translated. By 4 h after fertilization, more than 85% of the hybridizable H1 and H2B mRNA sequences are present on polysomes. Since no new synthesis of either of these sequences is detected over the first 5 h of development, H2B and H1 mRNAs probably follow the same pattern of control as does H3 mRNA, indicating a "histone specific" translational regulation. To see whether this regulation is also present in other sea urchin embryos, labeled DNA probes specific to H2B and H3 were hybridized to *L. pictus* polysomes. As in *S. purpuratus* very little H3 or H2B mRNA is present in the polysomal fraction 1 h after fertilization, yet by 3 h the majority of both mRNAs are polysomal. The results indicate that the pattern of translational regulation in *L. pictus* embryos is the same as in *S. purpuratus* embryos.

*Aspects of dogfish (*Mustelus canis*) lens differentiation.* SEYMOUR ZIGMAN, TERESA PAXHIA AND TERESA YULO (University of Rochester, Rochester, NY 14642).

In the ocular lens, epithelial cells, which line the anterior aspect of the lens subcapsularly in a single layer, divide and multiply and then migrate interiorly, where they change into elongated, non-nucleated and super-differentiated fiber cells. In dogfish lenses, several additional protein chains that were not previously synthesized in the epithelium were found to be synthesized in the fiber cells (*i.e.*, 65,000 and 17,000 daltons). The rate of protein synthesis in the fiber cells was only 5% of that in the epithelium, as determined by autoradiography using X-ray film, and by ^{14}C-amino acid *in vitro* incubation techniques using Econofluor liquid scintillation counting. The cell membranes of cortical fiber cells also become stabilized when they migrate into the nucleus. Slab gel polyacrylamide electrophoresis revealed that the membranes contain a heat-stable intrinsic polypeptide (26,000–27,000 daltons), but also many of the formerly soluble lens peptides that are bound irreversibly to them in the lens nucleus. The result is an attachment of high molecular weight aggregates to lens cell membranes, and enhanced light-scattering in the nucleus. Scanning electron microscopy of minced lens tissues showed that neither 8 *M* urea nor 1% SDS totally disrupted fiber cell membranes, but that the membranes were totally disrupted by 1% SDS plus dithiothreitol (50 m*M*). Such treatment released both intrinsic and extrinsic protein chains. Thus oxidative processes lead to binding of large quantities of proteins (via -SS bonds) to fiber cell membranes of the dogfish lens nucleus.

Support: N.E.I. and Research to Prevent Blindness, Inc. grants.

ECOLOGY

Algal extracellular products: kinetic analysis of their utilization by native microbial populations. WAYNE H. BELL AND DAVID M. GUTSTEIN (Hamilton College).

Heterotrophic bacterial populations in freshly obtained water samples from Vineyard Sound and Eel Pond were shown to possess enzyme-mediated transport systems for ^{14}C-labeled extracellular products derived from the algae *Skeletonema costatum, Thalassiosira pseudonana,* and *Dunaliella tertiolecta.* Bacterial transport systems functioned even though these algal species were absent from the water samples. However, kinetic analyses by the method of Bell (1980, *Limnol. Oceanogr.* **25:** 1007–1020) indicated that the bacterial transport and respiration of extracellular products were rate-limited by different compounds, different metabolic processes, or both. (The X-intercepts, or "$K_t + S_n$" for net transport and respiration lines failed to coincide.) In contrast, bacteria growing in the presence of a given algal species in continuous culture, or during a natural phytoplankton bloom, have extracellular products' uptake and respiration limited by the same enzyme-mediated transport system(s). (The X-intercepts for net transport and respiration lines are identical.) These results indicate that native bacteria have a diversity of metabolic systems, which allow them to use exogenous organic compounds at low concentrations from a variety of algal sources. But during algal bloom conditions, bacterial growth and metabolism become limited by the extracellular product(s) released by the dominant algal species.

This work was supported in part by the Steps Toward Independence Program of the Marine Biological Laboratory and by a faculty research grant from Hamilton College.

Limiting resources and the limits to reproduction in the "mud" snail Ilyanassa obsoleta *in Barnstable Harbor, Massachusetts.* G. A. BRENCHLEY (University of California, Irvine).

Ilyanassa obsoleta lays its eggs within capsules on solid surfaces in the intertidal zone. To study which substrates were preferred for capsule deposition, glass finger bowls (n = 27) in running sea water were used to contain four mud snails with a stone ($\sim$30 cm^2 area), peat of *Spartina* ($\sim$40 cm^2 area), and strands (20–25 cm lengths) of the introduced green alga *Codium.* More capsules (total 2519) were laid on glass walls (44%) and on *Codium* (40%) than on peat (14%). Rocks were rarely used (1%). A similar experiment involved compartments (n = 72) of plastic storage boxes with additional substrates present. Again, solid surfaces of the containers were preferred (70% of 9409 capsules) while those of rocks and "*Modiolus demissus*" shells were avoided (<1%). Of 2822 capsules laid on vegetation, *Codium* (37%), a filamentous brown alga (27%), and blades of *Zostera* (25%) were preferred over peat (9%) and blades of *Spartina* (2%).

Surfaces suitable for egg capsule deposition are rare in Barnstable Harbor (Pechenik 1978, *Biol. Bull.* **154:** 282–291). During the peak of reproduction (May–June), most preferred surfaces are occupied by the introduced periwinkle *Littorina littorea.* In field studies utilizing wooden clothes-pins as substrates, *Ilyanassa* emigrated from tide pools containing *Littorina* and consequently laid fewer egg capsules than in *Littorina*-removal pools (14 ± 17 versus 252 ± 49 capsules per clothes-pin, each n = 3). *Littorina*-free surfaces (plastic meshes, 10 × 30 cm with 0.6 cm openings), placed at three sites in June, had 2–3 orders of magnitude more capsules after 7 d than did the surrounding areas (152,850 ± 71,929 capsules per mesh, n = 5). *Ilyanassa* laid clusters of egg capsules up to 10 layers thick on strands of the preferred species, *Codium* and *Zostera*, presumably because surfaces lacking *Littorina* were so rare. These masses of capsules became easy prey for the introduced green crab *Carcinus maenas* and native hermit crab *Pagurus longicarpus*, and were also eaten by *Littorina.* Caging experiments revealed an average predation rate of 52% on masses of egg capsules in tide pools.

Supported by a Steps Towards Independence Fellowship, travel by Bio-Med funds from U.C., Irvine.

Use of internal growth bands for age determination in a population of Geukensia demissa *(Ribbed Mussel).* DIANE J. BROUSSEAU (Fairfield University).

Two methods used for age determination in bivalve molluscs involve counting (1) external annuli present on shell surfaces and (2) microscopic bands visible in acetate peels of cross-sections of molluscan shells. This study was designed to determine the feasibility of using each method to assess age and growth rate in a population of the ribbed mussel, *Geukensia* (=*Modiolus*) *demissa* from Westport, CT. Results from both methods indicate that *Geukensia demissa's* growth rate decreases with increasing age. However, internal growth bands appear to be more sensitive age indicators than external rings in the population studied. Out of 60 individual comparisons, only one-third of the individuals possessed the same number of internal and external shell bands. Of those remaining, 75% had fewer external rings than internal ones, suggesting that method 1 underestimates the age of older animals. This is expected since external rings become increasingly difficult to discriminate late in life. Estimates of longevity made from internal bands indicate that mussels from Westport, CT, live at least 11 years and probably longer.

I would especially like to thank J. W. Ropes (NMFS) for his assistance with the acetate peel technique. This work was in part supported by a grant from the Fairfield University Research Committee.

Seasonal feeding and distribution of Palaemonetes pugio *and* P. vulgaris *in Great Sippewissett salt marsh.* RANDY CHAMBERS (University of Massachusetts, Amherst).

Grass shrimp of the genus *Palaemonetes* were sampled monthly over the past year. *P. pugio* was typically taken in high marsh creeks; *P. vulgaris* was taken from low marsh creeks. *P. pugio* was the larger shrimp in every month, and during reproductive months female *P. pugio* brooded greater numbers of eggs than did *P. vulgaris*. No species differences in food items were observed (350 shrimp dissected), but gut contents varied with the seasonal abundance of prey. Adult shrimp grew and reproduced more when they ate more nematodes and polychaetes. Juvenile shrimp grew rapidly in July and August on diets of various small algae and crustaceans. In proportion to shrimp size, the second chelae of *P. vulgaris* were found to be significantly larger than those of *P. pugio* ($p < 0.05$). Laboratory experiments tested the behavior of selected paired shrimp in 500 ml containers (100 trials). Female shrimp dominated male-female interactions, using antennae and second chelae to physically displace males. For same-sex, same-species pairs, either no dominance was established (20 trials) or the larger shrimp was dominant (7 trials). For same-sex, different-species pairs, *P. vulgaris* established dominance in 21 of 28 trials. *P. vulgaris* is known to competitively displace *P. pugio* from preferred substrates. The mechanism of this displacement could be associated with the aggressive behavior of *P. vulgaris* described in these experiments.

The Effects of Food, Density, and Snail Size on the Floating Behavior in Hydrobia totteni (*Morrison*). GEORGE C. CHOW AND JEFFREY T. LIGHT (University of Colorado).

Floating rates of small (0.5–1.5 mm) and large (2.5–3.5 mm) snails were measured at densities of 1, 4, and 8 snails$\cdot$cm^{-2} under two food conditions of *Phaedactylum* diatom enriched sediment. In all 48 cases, increased food resulted in decreased overall floating. Small snails showed a higher percentage floating at greater densities, while large snails showed the opposite trend. At given densities and food, small snails floated more than large snails. Periodic fluctuations in floating over time were observed. Exploitative competition and crowding of snails can explain these laboratory observations. Daylight field observations during July 1981 showed no floating.

Interactions of the herbivore Eryhephala maritima *with salt marsh Chenopods.* CHARLOTTE M. COGSWELL (University of Connecticut) AND DEBORAH S. LERER.

The Chrysomelid beetle *Erynephala maritima* feeds on seven members of the Chenopodiaceae at Great Sippewissett Salt Marsh, Falmouth, MA. Tests of larval and adult feeding preferences among seven plant species show that *Salicornia europaea* is the most preferred food. It is followed by an intermediate preference group, *Salsola kali, Suaeda linearis, Salicornia bigelovii,* and *Atriplex patula*; and a low preference group, *S. virginica* and *Suaeda maritima*. In two-way comparisons of the *Salicornia* spp., including both control marsh and sewage sludge fertilized *S. europaea*, larvae and adults prefer feeding on fertilized *S. europaea*, followed by control *S. europaea, S. bigelovii,* and *S. virginica*. For *S. europaea* there is also a significant within-plant preference for young over old leaf tissue. The % wet weight or succulence of a *Salicornia* sp. is positively correlated with its feeding preference rank. *Salicornia* spp. % C, % N, C/N, and total soluble phenolics (determined by extracting 50°C dried plant tissue with 85% methanol and measuring absorbance at 324 nm) do not correlate with feeding preference ranks. *Salicornia* spp. have approximately 60% of their phenolic compounds in common, as determined by 2 dimensional paper chromatography (BAW 4:1:5 and 15% HOAc) on the methanol plant extracts rinsed with hexane. *S. bigelovii*, however, has two cinnamic acids not found in *S. europaea* or *S. virginica*.

Larval growth rates on *Salicornia* spp. and *Suaeda linearis* were determined by noting instar stage over time. Fertilized and control *Salicornia europaea*-reared larvae molt soonest and have the lowest mortality rates. *S. virginica-*, *S. bigelovii-*, and *Suaeda linearis*-reared larvae have longer molt times and higher mortality rates. *Salicornia bigelovii* gave the highest mortality rate and lowest growth rate, which might be explained by its unique cinnamic acids.

Deborah Lerer was supported by a grant from the Dept. of Botany, U. Mass, Graduate School.

The clearance rates of chlorophyll a *of the freshwater mussels,* Anodonta implicata *Say and* Elliptio complanata *(Lightfoot).* PAULA DAUKAS, BRUCE J. PETERSON (Marine Biological Laboratory) AND WILLIAM B. BOWDEN.

Laboratory measurements were made of the clearance rates of phytoplankton by *Elliptio complanata* (Lightfoot) and *Anodonta implicata* Say. Freshwater mussels from the North River (MA) were held in an aquarium containing water and sediments from the river. Clearance rates were calculated from the rate of decrease of *in vivo* chlorophyll fluorescence during 1–3 h experiments. Controls consisted of (1) the algal suspension with no mussels and (2) filtered water containing no chlorophyll and one mussel. This second control was used to detect any fluorescent substances released into the water by the animal. Clearance rates for *E. complanata* (dry body weights 0.65–7.45 g) ranged from 0.02–0.25 $l \cdot g^{-1} \cdot h^{-1}$. Rates were obtained for *A. implicata* (dry body weights 1.25–3.25 g) ranged from 0.05–0.30 $l \cdot g^{-1} \cdot h^{-1}$. Similar experiments with the marine blue mussel, *Mytilus edulis* L. (dry body weights 0.59–0.87 g) gave clearance rates of 2.5–4.2 $l \cdot g^{-1} \cdot h^{-1}$; much higher than those obtained with the freshwater species.

Preliminary experiments also measured the clearance rates of bacteria by freshwater mussels. Concentrations of cells were counted directly by epifluorescence after they were stained with Acridine Orange. Rates were dependant upon bacteria size. Mussels removed small bacterioplankton (4×10^{-3} to 3×10^{-2} μm^3), at negligible rates at the same time that they cleared phytoplankton at a rate of 0.21–0.31 $l \cdot g^{-1} \cdot h^{-1}$. The clearance rates of larger, filamentous bacteria (individual cell size approximately 1.8 μm^3) ranged from 0.41–0.78 $l \cdot g^{-1} \cdot h^{-1}$, nearly twice the rate of chlorophyll *a* removal by the same mussels.

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Interaction of mercury compounds with leucine transport in the intestine of winter and summer flounder. A. FARMANFARMAIAN, MARC EISENBERG, ROBIN SOCCI, VICTOR IANNACCONE, AND RAY HSIA (Rutgers University, Piscataway, NJ).

Mercury compounds released into coastal waters inhibit enzyme activity in various animal tissues. Less is known about the effect of heavy metal compounds on membrane transport. We have examined the interaction of mercuric chloride and methylmercuric chloride with the *in vitro* absorption of an essential amino acid by the intestinal tissue of winter flounder (*Pseudopleuronectes americanus*) and summer flounder (*Paralichthys dentatus*).

In the winter flounder, the uptake of 1 m*M* L-leucine from buffered fish Ringer over a 10 min period was inhibited 26% by 2- and 42% by 10-ppm Hg^{++} ($P < 0.05$). Incubation for 30 min gave essentially the same results. In a parallel series of experiments the uptake rate for summer flounder was significantly, $P < 0.05$, higher than winter flounder (3.77 vs 1.26 μmoles g^{-1} hr^{-1}) and inhibition by Hg^{++} was slightly higher (28% and 52% for 2- and 10-ppm respectively for a 10 min incubation). The above fish were collected from the polluted waters of Sandy Hook Bay, NJ. For comparison, we examined intestinal leucine uptake in fish collected from the clean waters of Brigantine Inlet, NJ, and Buzzards Bay, MA. At 0.25 m*M* leucine and 10 min incubation, the fish from clean waters had a higher rate of absorption than fish from Sandy Hook Bay. Furthermore, uptake in fish from polluted waters was more sensitive to low levels of Hg^{++} (significant, $P < 0.05$, inhibition at 0.5 and 1.0 ppm) than fish from clean waters. In the latter group significant, $P < 0.05$, inhibition was observed at 10 ppm and above. Taken together, these data imply that polluted waters reduce the capacity to absorb amino acids from food and that adding low doses of Hg^{++} to the gut accentuates this effect. By contrast, CH_3Hg^+ does not significantly, $P < 0.05$, inhibit leucine uptake at 1 and 10 ppm and only shows a 33% inhibition at 20 ppm. This implies that the charges on the divalent Hg^{++} ion are essential for the effective binding and inhibition of the leucine carrier.

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Natural gas from salt marsh biomass. Rodney M. Fujita (Boston University Marine Program, Marine Biological Laboratory).

Applying sewage sludge on plots in Great Sippewissett salt marsh (north of Woods Hole, Massachusetts) increases production of the dominant grass, *Spartina alterniflora*, and assimilation of nutrients and contaminants in the sludge. To assess the potential of salt marshes for treating waste and producing biomass for conversion to energy and for nutrient recycling, the suitability of indigenous plants, algae, and cultivated algae as substrates for anaerobic methane (natural gas) production must be determined. *Spartina alterniflora* from unfertilized sites and from plots treated with processed sewage sludge (7.5 g N, 4.5 g P_2O_5, and 3.0 g $K_2O \cdot m^{-2} \cdot week^{-1}$) was chopped and loaded into anaerobic 800-ml-capacity digesters containing salt-marsh sediment and seawater. Unfertilized *Enteromorpha* sp., a common green alga, was loaded into similar 400-ml-capacity digesters. The digesters were kept at 35°C. Total gas production and methane content were measured. After an initial delay of 13 days, unfertilized *Spartina* yielded 0.26 l of biogas$\cdot g^{-1}$ of volatile solids (v.s.) loaded at 72% methane over 48 days. After an initial delay of 8 days, fertilized *Spartina* yielded 0.11 l$\cdot$g v.s.$^{-1}$ at 65% methane over 21 days. Heavy metals assimilated by the fertilized *Spartina* may have inhibited gas production. After an initial delay of 2 days, *Enteromorpha* yielded 0.21 l$\cdot$g v.s.$^{-1}$ at 74% methane over 32 days.

Mechanisms of coexistance in two species of Fundulus. Gary D. Hals. Capital University, Columbus, Ohio.

Fundulus heteroclitus and *Fundulus majalis* co-occur in large numbers in salt marshes. The differences in morphology lead to a difference in food items taken. Laboratory experiments that showed *F. majalis* take more prey at any given depth in coarse or muddy sand. Partitioning through feeding substrate preferences tested showed no preference in *F. heteroclitus* for coarse or muddy sand, and a significant trend towards preference of course sand in *F. majalis*. Previous research has shown that *F. heteroclitus* gets most of its food from *Spartina alterniflora* beds where muddy sands are found, and may exhibit no preference in the lab due to experimental error. Preference for coarse sand in *F. majalis* is supported by large numbers (average of 176 individuals in coarse sand, 99 in muddy sand) and biomass (average of 0.37 g in coarse and 0.14 g in muddy sand) of infauna. *F. majalis* was also shown to take more prey from coarse sand at levels below 3 cm. These results support that morphology directly contributes to coexistance through food partitioning. They also suggest that competition could be further reduced by partial preferences in feeding areas.

Selective pressures exerted by sewage sludge fertilization of chemoheterotrophic bacterial communities in the Great Sippewissett Marsh. Nancy V. Hamlett (Department of Biological Sciences, Towson State University, Baltimore, MD 21204), Jeanne S. Poindexter, and William S. Reznikoff.

The purpose of this study was to examine the effects of fertilization with sewage sludge on aerobic chemoheterotrophic bacterial sediment communities in the Great Sippewissett Marsh. Experimental plots (10 m radius, drained by a single stream) have been fertilized with sludge-containing fertilizer contaminated with heavy metals including mercury. Bacteria from cores of the upper 2 cm of sediment were enumerated by plate counts on a yeast extract-peptone-seawater medium. Bacterial colony-forming units were more numerous in sludge-fertilized plots and in plots fertilized with urea and phosphate than in control plots. The proportion of mercury-resistant bacteria was increased in sludge-fertilized but not in urea-treated plots. By replica plating on antibiotic and metal-containing media, a strong correlation between mercury resistance and resistance to other metals and antibiotics was detected among isolates from control and urea-treated plots. Among isolates from sludge-fertilized plots, the correlation was less pronounced because mercury-sensitive isolates also showed substantial antibiotic resistance. By gel electrophoresis, plasmids have been detected in some, but not all, mercury-resistant isolates. Predominant colony types were also altered in sludge-fertilized plots. In control and urea-treated plots, no one type accounted for more than a few percent of the total colonies. Two Pseudomonas-like bacteria accounted for 70% of the mercury-resistant colonies. In sludge-fertilized plots, a Cytophaga-like bacterium accounted for 35% of the total and 50% of the mercury-resistant colonies. Spore-formers and a third pseudomonad made up another 15% of the total and 20% of the mercury-resistant colonies. The predominance of the Cytophaga-like bacterium may relate to its ability to use for nutrients carcasses of bacteria in sludge. Complex selective factors including metal contamination and the form of nutrients in sludge apparently have altered the structure of this portion of the bacterial sediment community.

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An assessment by microautoradiograhy of microbial activity in Spartina *litter in flow-through percolators.* JOHN HELFRICH, JOHN E. HOBBIE, AND ANDREW C. MARINUCCI (Marine Biological Laboratory).

To directly measure microbial activity in decomposing *Spartina* litter, we have adapted the autoradiographic technique of L. -A. Meyer-Reil (1978, *Appl. Env. Microb.* **36:** 506–512) as modified by Paul Tabor (Naval Research Laboratory, Washington, D. C.). We have applied this technique to effluent and litter from flow-through percolators, laboratory microcosms for the study of decomposition processes.

Samples taken throughout a 9 week period were radio-labeled with a tritiated amino acid mixture (New England Nuclear) at the rate of 2.0 μCi per 10 ml of effluent, or per 100 mg (wet wt) of litter with 5 ml of added filtered seawater. Litter samples were homogenized with an additional 35 ml of filtered seawater at 22,000 rpm for 5 min. Suitable amounts of homogenate or effluent were gently filtered through 0.2 μm Nuclepore filters. In total darkness, filters were placed face down on microscope slides coated with Kodak NTB-2 liquid emulsion. After 4 days of exposure, slides were photographically developed, stained with Acridine Orange, and destained in a citrate buffer series. Filters were carefully peeled from the slides, leaving the microbes attached to the emulsion. Counts were made of total cells and of active cells associated with silver grains, using epifluorescence and phase-contrast microscopy.

In effluent samples, total cells ($\times 10^6 \mathrm{ml}^{-1}$) were 87 after 1 day, decreasing to 5.2 after 7 days and to 0.25 after 53 days. Active cells ($\times 10^6 \mathrm{ml}^{-1}$) in the same periods were 15, 2.4, and 0.12, while percent of cells active were 24, 44 and 48. In the litter, total cells ($\times 10^9$ gram dry weight^{-1}) were 29 after 5 days, 34 after 28 days, and 45 after 53 days. Active cells in the same periods were 9.5, 6.5, and 4.5; percent active were 33, 19, and 10.5.

We speculate that the initial effluent peak was stimulated by leaching of soluble nutrients. Colonization of the litter was similar to that in litterbags in the field. Decrease of active cells may reflect specialization of the microbial community for the breakdown of refractory plant compounds.

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Caudal regeneration as a measure of senescence in Capitella *sp. I (Polychaeta).* SUSAN DOUGLAS HILL (Michigan State University) AND JUDITH GRASSLE.

Capitella sp. I is the most opportunistic of the sibling species in this genus, formerly known as *Capitella capitata.* The generation time at 26°C is approximately 4 weeks, and individual females produce a series of broods of large, yolky eggs in quick succession. Laboratory and field populations undergo the classical boom and crash cycle in the presence of abundant food. To understand sudden population declines, it would be useful to have an independent measure of senescence that could be related to an age-specific decline in fecundity. We have examined the relationship between regeneration rate, age, and the level of reproductive effort in a population of closely related individuals. Worm caudal segments were amputated at the 20th abdominal segment. A new pygidium formed within 48 h, followed by a growth zone composed of very small segments. Regenerated segments could usually be distinguished for 5 weeks by an abrupt change in segment length at the amputation level, and by the increased transparency and lack of pigmentation in the regenerated portion. Amputated segments were counted to determine body length; worms were examined weekly and regenerative segments counted. In some females the level of reproductive effort was reduced by preventing mating; other females were allowed to mate and produced five broods. Eggs per brood were counted as a measure of age-specific fecundity. Males were either kept at high density or isolated. The isolated males quickly became functional hermaphrodites. Preliminary results indicate no significant difference between the 5-week regenerative capabilities of young males and females, indicating that the two processes of oogenesis and regeneration can occur simultaneously without reducing the number of segments regenerated. Males that switched to being hermaphrodites, *i.e.* initiated oogenesis, regenerated significantly fewer segments. Both regenerating males and females possess significantly more segments than non-regenerating animals of comparable age.

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Feeding preferences and competitive herbivory studies on two species of periwinkles, Littorina obtusata *and* Littorina littorea. JUDY HUNTER (Auburn University, Auburn, Alabama 36830)

Feeding preference studies and competitive herbivory experiments were conducted with the native New England periwinkle, *Littorina obtusata*, to determine preferred algal species and the effects of the introduced *Littorina littorea* upon *L. obtusata* food consumption. European studies on *L. obtusata* show a preference of this species for fucoid algae for feeding and spawning. However, *L. littorea* does not regularly eat fucoids, as they contain noxious inhibitory chemicals.

Four species of algae were tested using both whole plants and a medium consisting of homogenized algae and agar. When whole plants were available, *L. obtusata* consumed the green alga *Ulva lactuca* as well as the fucoids *Ascophyllum nodosum* and *Fucus* sp. When offered equal amounts of *Ulva* and *Ascophyllum* in agar media, *L. obtusata* preferred *Ascophyllum*; when offered *Ulva* and *Fucus* in agar media, they showed only a slight preference for *Fucus*. On separate plates, when *Fucus* and *Ascophyllum* were offered in a 2:1 ratio to *Ulva*, the fucoids were overwhelmingly preferred. On the same plate, in three-way choice experiments on agar media, *L. obtusata* preferred *Fucus* and *Ascophyllum* when offered these two in equal amounts with *Ulva*. In competition experiments, when one individual each of the two congeners was placed together on an *Ulva* agar media plate, *L. littorea* suppressed the feeding of *L. obtusata* significantly. Upon removal of *L. littorea, L. obtusata* feeding increased greatly.

Persistence and distribution of marine vibrios in the hardshell clam, Mercenaria mercenaria. HEIDI B. KAPLAN AND E. P. GREENBERG (Cornell University, Ithaca, NY).

The occurrence of marine vibrios in *Mercenaria mercenaria* was investigated in both laboratory and field studies. In the laboratory, *M. mercenaria* was inoculated with strains of *Vibrio parahaemolyticus*, the luminous bacterium *Vibrio harveyi*, and *Escherichia coli* by incubation for 2 h in seawater containing each of these bacterial species at concentrations of 1000 cells/ml. Subsequently, the *M. mercenaria* specimens were transferred to an artificial purification chamber (a tank through which UV-sterilized seawater was continually flowing). As determined by differential plating techniques, the number of *E. coli* in *M. mercenaria* tissues decreased approximately 100-fold within 24 h while the numbers of *V. parahaemolyticus* and *V. harveyi* showed little or no decrease within this period. After 72 h, the numbers of the two species of *Vibrio* had decreased approximately tenfold.

The natural abundances of luminous vibrios and organisms presumptively identified as *V. parahaemolyticus*, from seawater and tissues of *M. mercenaria*, were studied. The sampling sites were Eel Pond, MA, a polluted harbor closed to shellfishing, and Great Pond, MA, an area open to shellfishing. Neither bacterial group was detected during the winter of 1980-81. With rare exceptions, both groups were isolated from seawater and from tissues of *M. mercenaria* throughout the late spring and summer. For any sampling period, the numbers of each of the groups of vibrios appeared to be approximately 100 times greater per gram of *M. mercenaria* tissue than per ml of seawater. Population densities often fluctuated markedly between sampling times. For example, 19 luminous vibrios per ml of Eel Pond seawater were detected on June 26; and on July 8, only 8 luminous vibrios per ml of Eel Pond seawater were detected. There were no significant differences in results obtained from either sampling site. There appeared to be some correlation between the numbers of luminous vibrios detected in a sample and the numbers of presumptive *V. parahaemolyticus* detected; luminous vibrios occurred at densities approximately tenfold greater than presumptive *V. parahaemolyticus*.

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Settlement, growth and survival of the scyphistoma stage of Aurelia aurita. SUSAN L. KEEN (Division of Biological Sciences, University of Michigan. Ann Arbor, Michigan 48109.)

Larvae of the jellyfish *Aurelia aurita* occur in 2 overlapping pulses in Eel Pond, Woods Hole, MA, during the summer. Settling plates, each composed of 36 unglazed ceramic tiles, collected larvae. Within one plate, 7 tiles (three high density, $n \simeq 54$; two intermediate, $n \simeq 20$; two low, $n \simeq 1$) were examined approximately every 5 days: polyp sizes, growth, number of buds and density of recruits were recorded in relation to initial densities.

Recruitment was correlated with conspecific presence and was greatest on high density tiles. Budding occurred first on these tiles, as did larvae of the second cohort. Growth rates after 29 days were highest on high density tiles (0.05 mm/day) and declined with time. Growth rates declined with time on all tiles and differences in growth rates, in time of budding, and in appearance of the second cohort probably reflect earlier recruitment on high density areas.

After 48 days, frequency distributions of high density tiles ($n \simeq 230$) have the same shape as those from intermediate densities ($n \simeq 92$). The same percentage of polyps bud in high and medium densities, but percentages change with time. Individuals and their buds represent a single genotype and no size differences exist between densities. After 34 days, budding polyps averaged 0.392 ± 0.107 mm, larger than polyps which did not bud.

Polyps in the second cohort grew more slowly (0.01 mm/day) than those in the first cohort and occupied a variety of surfaces. After 40 days, 7% (195/2631) of polyps were on erect bryozoan branches (*Bugula*) and ate bryozoan zooids.

Reciprocal transplants between high and low density tiles resulted in loss of polyps on high density tiles and increased numbers on low density tiles. Position effects were significant. Growth, size per genotype, and percent of individuals budding are independent of density. Polyp aggregation may result from larval colonization of areas in the best positions within a settling plate.

Contextual relationships in food webs involving meiofauna. II. Selective enrichment experiments and differential cropping. JOHN J. LEE (City College Of City University of New York), ANAISA DELGADO-HYLAND, AND MONICA J. LEE.

The total potential energy present in *Spartina* detritus is channeled relatively slowly into secondary production by the actions of microorganisms and the food webs linked to them. Among the most numerous animals in the detrital food web are the micro- and meiofauna. They are important energy channels because of their rapid growth rates and high intrinsic rates of increase. Evidence from gnotobiotic studies has suggested that many of these small animals are quite specialized in their nutritional requirements, and that the most successful have potential for optimizing their nutritional and energetic needs by selectively consuming microfloral species.

For the past three summers, we have been conducting laboratory and field experiments aimed at clarifying relationships between herbivorous meiofauna and microfloral assemblages. In all experiments we were interested in whether microfloral assemblages selectively control meiofaunal abundances and fecundity and whether meiofauna selectively alter the population structure of the diatom component of the microfloral assemblages. Selected meiofauna and natural or artificial microfloral assemblages were inoculated into tissue culture flasks and were incubated *in situ* in Greater Sippewissett salt marsh. Windows covered with Teflon filters (3 μm) were cut into the tissue culture flasks, allowing free passage of sea water while still retaining the experimental organisms.

Evidence from the laboratory and field studies just concluded suggests that major pathways of energy flow through the meiofaunal assemblage at any particular time depend upon the matches of these animals with appropriate food species.

More than 1.5×10^5 individual diatoms from experimental flasks have been enumerated. As might by anticipated, quite a number of taxonomic problems have arisen which will be resolved by description of new species of varieties. Q and R mode cluster analytical techniques will be used to compare the successional trajectories of the diatom assemblages in replicate control and experimental flasks with each other, and with natural assemblages outside the flasks.

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Response of decomposition and fungal biomass of Spartina alterniflora *litter in a flow-through percolator to initial plant nitrogen.* ANDREW C. MARINUCCI (Marine Biological Laboratory), JOHN HELFRICH, AND JOHN E. HOBBIE.

Microbial decomposition of *Spartina alterniflora* litter in the salt marsh is pivotal to that ecosystem. Laboratory simulation of this process in a flow-through percolator allows continuous monitoring of changes in the major chemical components of the litter (Marinucci, A. C., 1981, Ph.D. Dissertation, Rutgers University). In the present study, we examined the effects of different initial litter nitrogen (ILN) levels of *S. alterniflora* on CO_2 evolution, ash-free dry weight (AFDW), and nitrogen (N) changes. We also examined final fungal biomass after decomposition at 20°C for 56 days in this percolator. ILN levels were 0.85, 0.95, 1.22, and 1.33% N/AFDW as a result of increased levels of fertilizer applied to the salt marsh (Valiela, I., 1975, *J. Appl. Ecol* **12:** 973–982).

CO_2 evolution increased directly with increased ILN. The AFDW in turn decreased with increased

ILN. The concentration of nitrogen in the decomposed litter increased relative in ILN as ILN increased and ranged from 82% in 0.84% N litter to 103% in 1.33% N litter.

Fungal hyphae were measured directly by examining agar films with phase contrast microscopy and applying the appropriate corrections (Berg and Söderström 1979, *Soil Biol. Biochem.* **11:** 339–341). Results also had to be corrected for sample size. Final calculated hyphal biomass did not show any significant dependence on ILN, but did range between 2.3 and 3.0% of the AFDW of the litter. The percentage of final litter nitrogen that resided in the fungal hyphae ranged between 28 and 52% and was inversely related in the ILN.

This work was sponsored by the National Science Foundation grant NSF-DEB-79-05127.

The distributional ecology of two Littorinid snails in the Great Sippewissett salt marsh. KIMBERLEY S. MERCURIO (Marine Biological Laboratory).

In the Great Sippewissett salt marsh, *Littorina littorea* occurs in mud flats and in the lower portions of *Spartina alterniflora* stalks, while *Littorina saxatilis* is found commonly on the higher portions of the *Spartina* blades. Differences in morphology (color, shape, size, and stability) and physiology (internal and lethal temperatures) were examined in order to identify factors that may contribute to these microdistributional patterns.

Littorina saxatilis tolerated greater lethal temperatures than *L. littorea.* This was also determined earlier by McMahon and Russell-Hunter for the same species on rocky shores: 1977, *Biol. Bull.,* **152:** 182–198. Internal temperatures were found to be slightly lower in *L. saxatilis* than in *L. littorea* in laboratory and field experiments with small sample sizes.

Various morphological features permit *L. saxatilis* (L.s.) to tolerate, more than *L. littorea* (L.l.), physical stresses that occur at greater heights on the *Spartina* stalks. These features include light coloration (L.s., yellow; L.l., brown) reflecting radiation; less surface area/volume measured in shell height (L.s., mean = 8.7 mm; L.l., 13.8 mm); smaller aperature, decreasing desiccation (L.s., mean = 4.8 mm; L.l., 10.3 mm), a broader posterior stability angle (L.s., mean = 24.9°; L.l., 19.7°) and a narrower lateral stability (L.s., mean = 16.1°; L.l., 13.0°) preventing dislodgement from the stalks. The absence of *L. saxatilis* from the lower intertidal, such as the mud flats, may in part be due to competitive processes resulting from the introduction of *L. littorea* in North America in the 1800's.

Interaction of ferulic acid, nitrogen, pH, and salinity on feeding by the salt marsh snail, Melampus bidentatus. CAROL S. RIETSMA (State University of New York at New Paltz).

The concentration of ferulic acid, a secondary plant substance found in detritus of *Spartina alterniflora,* decreases with age of the detritus. When ferulic acid is added to agar suspensions of naturally aged, 9-month-old detritus, it inhibits feeding by *Melampus bidentatus* (Valiela *et al.,* 1979, *Nature* **280:** 55–57). In further work, nitrogen content, pH, and salinity of detritus have been found to alter this inhibition.

To investigate the interaction of ferulic acid content, nitrogen content, pH, and salinity of detritus, these variables were manipulated in agar suspensions of 9-month-old detritus. Snails were allowed to feed on the suspensions in petri dishes with four compartments. Only two treatment combinations were included in each dish. Feeding was assessed by counting the number of feeding marks left by the snails on the surface of the suspensions.

Feeding activity was lower on suspensions with 8.5 mg ferulic acid/g dry weight detritus than on those with 1 mg. This inhibition was reduced or eliminated by added protein-nitrogen. When ferulic acid was added at an inhibitory concentration of 8.5 mg, feeding activity was lower on suspensions lacking added protein-nitrogen than on those containing it. Ferulic acid may be bound by the added protein-nitrogen and inactivated as a feeding deterrent. Varying salinity and pH had little or no effect on the interaction of ferulic acid and nitrogen. Deionized water, however, decreased overall feeding activity.

Nitrogen content of detritus is therefore the primary cue for feeding by *Melampus bidentatus.* It is followed by ferulic acid content, pH, and salinity.

The role of nitrate respiration among chemoheterotrophs in the Sippewisset algal mat. JAAP VANRIJN, RICHARD JURICK, ANN CASTELFRANCO, AND JEANNE POINDEXTER (Public Health Research Inst.).

Principally due to microbial denitrification, the nitrogen limited Sippewisset Marsh is a net exportor of nitrogen. These studies were undertaken to determine whether nitrate respiration gave denitrifying bacteria a competitive advantage over strict aerobes.

A chemostat growth vessel was inoculated with supernatent of centrifuged low-marsh-mat surface scrapings and interstitial water. Medium containing lactate, NH_4Cl, $FeNH_4C_6H_5O_7$, phosphate and 75% seawater was delivered at $D = 0.25\ h^{-1}$. After 18 volume changes, three bacterial types were isolated that failed to grow in nitrate agar shake tubes. One third of the chemostat culture was used to reinoculate the growth vessel. The same medium plus $NaNo_3$ was delivered for 22 volume changes under a nitrogen atmosphere. Five isolates from this culture grew and produced gas in nitrate agar shake tubes.

One pseudomonad-like, polarly flagellated bacterial isolate from each group was studied further. Both had aerobic doubling times of about 2 h, but only the second isolate grew anaerobically, and only in the presence of nitrate, with a doubling time of 4.5 h. These isolates were inoculated at equal densities into the chemostat vessel and provided with the lactate-nitrate medium at $D = 0.167\ h^{-1}$. To simulate the diurnal variation in oxygen content in the algal mat, the population was allowed to reach steady-state aerobically, the atmosphere was then shifted to nitrogen for several volume changes; finally air was reintroduced. The strict aerobe rapidly came to predominance during each aerobic phase; the denitrifier predominated only during the anaerobic phase.

These studies imply that nitrate respiration contributes to diversity among the chemoheterotrophic bacteria of the algal mat. The development of highly competitive aerobes appears to be favored during the day, while nitrate respiration allows continued, although slower, development of denitrifiers whenever oxygenic photosynthesis declines.

This research was supported by NASA grant NAGW-216 and grants from the Foundation for Microbiology.

Intraspecific competition in the periwinkle, Littorina littorea. ROBERT M. SHERRELL.

The snail *Littorina littorea*, a major herbivore in New England rocky intertidal habitats, is found at high densities in areas devoid of macroscopic algae. Growth rates were measured at controlled densities to determine the extent to which periwinkles compete intraspecifically on substrates supporting varying quantities of microscopic algae. Wire mesh cages (8 × 8 × 2.5 cm) were attached to the surface of wooden pilings at mid-tide level. Size-matched snails were introduced at densities of 0, 2, 5, and 10 individuals per cage. Three food levels were established: The unmanipulated standing crop served as a middle level, scraped pilings as a low level, and pilings fertilized daily as a high food level. After 19 days, new shell deposition was measured as an index of growth rate. To determine quantity of food remaining, samples of the piling surface in each cage were analyzed for chlorophyll *a* content, a relative measure of algal biomass. Samples from unused pilings controlled for effect of caging on ambient algal crop. A parallel laboratory experiment enclosed snails in perforated plastic boxes in a sea water table and maintained three food levels by feeding measured amount of *Ulva lactuca*.

At all food levels in the field and in the laboratory, growth rate and food remaining generally decreased with increased snail density. Growth rates at lower densities were proportional to food level. At highest density, snails' growth was minimal at all food levels, suggesting physical or chemical interactions inhibiting growth independent of food supply. Little depletion of food occurred in high-food, high-density cages, further indicating a crowding effect. Caging had no effect on standing algal crop. *Littorina littorea* competes intraspecifically for food. High densities may inhibit feeding regardless of food supply.

The response of benthic diatoms to the exclusion of macroconsumers. WENDY WAGNER (Hanover College, Hanover, IN) AND KENNETH FOREMAN.

We assessed the effect of grazing and bioturbation by fish (*Fundulus heteroclitus* and *F. majalis*) and crabs (*Uca pugnax*) on benthic diatom assemblages by excluding these macroconsumers from half-square-meter areas in two salt marsh creeks. One cage with 5 mm mesh was placed in each creek in mid-July. After 30 days, sediments in caged and uncaged areas were sampled. Sediments were also collected from an identical cage which had been in place in one of the creeks for 14 months. Abundance, species composition, and sizes of diatoms within the community were measured in both caged and uncaged areas.

A substantial increase in diatom density was observed in 30-day and year-old cages, averaging 23,000,000 diatoms per cc sediment and 37,000,000 diatoms per cc sediment, respectively. Uncaged sediments contained 14,000,000 diatoms per cc. Numerically abundant species in all locales included *Fragilaria construens* (30%), *Achnanthes hauckiana* (13%), *Navicula diserta* (5–11%), *Melosira nummuloides* (4–5%), and *Navicula cryptocephala* (2–4%). No significant shift in the composition of dominant species was discerned after caging, although a general trend towards larger species was observed: Diatom species above 50μ comprised 11% of the community in uncaged areas and 20% in areas caged for 30 days. Mean sizes of individuals within a species also increased. The mean frustule length of *Gyrosigma balticum* climbed from an initial 420μ to 432μ to 445μ in uncaged, 30 day, and one year cages, respectively, and *Pleurosigma angulatum* increased from 205μ to 225μ to 240μ in uncaged, 30 day, and one-year cages (Paired t test, $p < 0.01$).

The trend towards increased size after macroconsumer removal suggests that cages are providing a partial refuge from grazing, favoring larger diatom species. These results indicate that fish and crabs are influential in regulating the abundance and size distributions of diatoms within the benthic diatom community.

Recruitment of salt marsh macrobenthos. WENDY WILTSE (Williams College-Mystic Seaport Program, Mystic, CT).

Preference of benthic macroinvertebrates for sediments of control- and sewage-fertilized tidal creeks was examined at Great Sippewissett Marsh, MA. Recruitment of macrofauna was compared in vials (1.3 cm^2 surface area) containing four different sediments: natural control, natural fertilized, azoic control, and azoic fertilized. Natural sediments were intact cores from control and sewage-fertilized creeks. Azoic sediments were sieved, frozen, and thawed twice to homogenize sediments and kill organisms. Fertilized sediments had more nitrogen and carbon than controls. Vials were randomized in ranks and placed in a control creek for 5 and 13 days.

The highest number of organisms recruited into natural control sediments. There were no differences between natural fertilized and azoic sediments. Larvae of *Streblospio benedicti* settled preferentially in natural control sediments, indicating that selective larval settlement accounts for the predominance of *S. benedicti* in control creeks. The preference of larvae for natural control over azoic control sediments suggests that biological or surficial properties are important cues for settlement.

Other benthic animals were highly mobile and recruited into vials as juveniles and adults. *Hydrobia* sp., *Nematostella vectensis, Paranais litoralis,* and *Nereis succinea* recruited into ≥25% of the azoic vials but did not show significant preference among sediment types.

Preliminary observations of bacteria and shipworms (Bivalvia: Teredinidae). ANSON E. WRIGHT, COLLEEN M. CAVANAUGH (Harvard University). SCOTT M. GALLAGER, ROGER MANN, AND RUTH D. TURNER.

It has been inferred that bacteria are important in the nutrition of shipworms. Here we report (1) preliminary observations of the bacteria found in the fluid in the shipworm burrow and (2) the results of experiments to quantify shipworms' filtration of bacteria from ambient seawater. In an experiment using $10^{-4}M$ rhodamine dye in tank water, we found no detectable dye in the burrow of *Lyrodus pedicellatus* Quatrefages after 2 days and little after 6 days, indicating slow exchange between the mantle cavity and the burrow fluid. Bacterial numbers in the burrow fluid of *L. pedicellatus* and *Bankia gouldi* Bartsch ranged from 1.6 to $7.1 \times 10^7 \cdot ml^{-1}$ ($n = 6$), as determined by direct counts using fluorescence microscopy. The bacteria were larger, morphologically distinct, and two orders of magnitude more numerous than those in the water from the seawater-table. They included large rods (up to 2 μm × 6 μm), spirilla, and long filaments. Spirochaetes were isolated anaerobically from the burrow fluid, and cellulolytic activity was observed in both aerobic and anaerobic enrichments for cellulose degraders. Although shipworms have been shown to retain close to 100% of phytoplankton greater than 4 μm in diameter, *L. pedicellatus* does not appear to retain more than 2–4% of bacterial cells of 1 μm or less.

Our results suggest that the bacteria in the burrow may contribute to the utilization of cellulose by *L. pedicellatus* and *B. gouldi.* It has been suggested that wood does not supply the total nitrogen requirements of shipworms. Whether these bacteria contribute to the nitrogen nutrition of shipworms has yet to be determined.

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Optimal foraging in the cunner, Tautogolabrus adspersus. DAVID L. WYNES (Dept. of Environmental Science, Nichols College, Dudley, Mass. 01570).

The relationship of prey size to strike and handling times was examined for three size classes of cunners, *Tautogolabrus adspersus.* Strike time tended to decrease and handling time to increase with increasing prey size. Capture times (sum of strike and handling times) were used to determine optimal prey size (the lowest capture time/benefit ratio). Prey (amphipods) were measured in milligrams dry weight in calculating this ratio. Optimal prey sizes were skewed toward the maximum prey size that a fish could consume. Small fish (5.0–6.0 cm total length, TL) consumed prey ranging from 4–10 mm with an optimal size of 9–10 mm. Medium fish (7.0–8.0 cm TL) consumed prey ranging from 4–15 mm with optimal sizes in the 9–15 mm range, while large fish (9.5–11.5 cm TL) consumed prey 5–19 mm in length with amphipods of 12–19 mm optimal.

Prey choice tests showed that all three sizes of fish preferred prey sizes predicted by optimal foraging theory. These tests also showed that preferred prey sizes differed significantly between size classes of fish.

FERTILIZATION AND REPRODUCTION

Effect of gossypol on the production and utilization of ATP by sea urchin spermatozoa. O. ADEYEMO, C. Y. CHANG, S. S. KOIDE, AND S. J. SEGAL. (Population Council, Rockefeller University, New York)

Gossypol inhibits human sperm motility; the effect is correlated with a fall in the level of ATP. The present study was conducted to determine whether or not gossypol influences mitochondrial enzymatic systems regulating ATP synthesis and consumption in sea urchin sperm. The enzymes studied were pyruvate dehydrogenase and ATPases.

A mitochondria fraction was prepared from sperm of *Arbacia punctulata.* Pyruvate dehydrogenase activity was determined by measuring $^{14}CO_2$ liberated from 1-^{14}C-pyruvate (Leiter *et al., J. Biol. Chem.* **253**: 2716; 1978). The enzymatic activity of mitochondria incubated with gossypol at concentrations of 10, 20, and 50 μM decreased by 55, 70, and 87% of control value, respectively. This suggests that a cause of the reported fall in ATP level induced by gossypol is the inhibition of pyruvate dehydrogenase activity and, consequently, ATP synthesis.

Mitochondria fraction prepared from sperm of *Hemicentrotus pulcherrius* (obtained by C. Y. Chang at the Institute of Oceanography, Tsingtao, China) was assayed for Mg^{2+}- and Na^+, K^+-dependent ATPase activities.

Activities of both ATPases were reduced when obtained from sperm treated with gossypol at concentrations of 5 μM and higher. The decrease in ATPase activity was proportional to gossypol concentrations used. The finding that gossypol inhibits ATPase activity suggests that the observed inhibition of sperm motility can be attributed, in part, to impairment in the utilization of ATP as a source of energy.

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Hydrogen peroxide blocks polyspermy in Arbacia *by affecting sperm motility.* C. ARANOW, M. EISEN, M. ZIMMERMAN, W. TROLL (NYU Medical Center).

Polyspermy is a potential hazard faced by all eggs fertilized in the presence of a high sperm/egg ratio. Viable offspring cannot result from eggs fertilized by more than one sperm. Many cells, including mammalian lymphoid cells, respond to activation by a variety of agents by excreting hydrogen peroxide. The sea urchin egg excretes H_2O_2 immediately after the first sperm fertilizes the egg. This could be a mechanism employed by *Arbacia punctulata* to prevent polyspermy. We have studied the effects of endogenous and exogenous on H_2O_2 on polyspermy, as well as of agents that destroy or reverse the effects of H_2O_2 on cells. The egg's hydrogen peroxide response is inhibited by protease inhibitors and retinol. These agents as well as catalase, an enzyme converting H_2O_2 to oxygen and water, cause polyspermy. We now report that H_2O_2 added to sperm in 10^{-3}–$10^{-6}M$ concentrations causes a change in motility and a decrease in fertilization of eggs. Sensitivity to changes in peroxide concentration appears to be greatest in the 10^{-4}–$10^{-5}M$ range. The action of H_2O_2 is reversed by addition of the S-S reducing agent dithiothreitol, causing reactivation of movement and fertilizing capacity. Mercaptoethanol and reduced glutathione do not reactivate H_2O_2 inactivated sperm. Two heme enzymes, catalase and horseradish peroxidase have opposite effects on the H_2O_2 action on sperm. Catalase, probably by blocking H_2O_2 inactivation, is capable of activating sperm. This reactivation of sperm indicates that inactivation by H_2O_2 may be temporary, retarding the motion of other sperm long enough for the fertilization membrane to be raised.

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Delivery of gossypol by liposomes: inhibition of sperm motility. Mario Burgos, Judith L. Fridovich, Gerald Weissman, and Sheldon J. Segal (Rockefeller Foundation, New York).

Gossypol (gp) is a polyphenolic aldehyde extracted from cottonseed. When administered to men, it suppresses the motility of epididymal sperm and interferes with spermatogenesis. Systemic doses required to achieve these effects may be greater than a theoretical minimal effective dose because of the tendency of the molecule to bind to serum proteins. Previous studies establish that doses of 10–100 μM/ml, when added to the ambient sea water, inhibit the motility of the sperm of *Arbacia punctulata* (Burgos *et al.* 1980, *Bio. Bull.* **159:** 469). The desirability of reducing the dose required to achieve biological activity prompted efforts to deliver gp in a manner that would protect against protein binding and inactivation, *i.e.*, through incorporation into the lipid phase of multilamellar vesicles (liposomes). Liposomes containing gp were prepared by adding 10% or 20% by mole gp to a chloroform solution containing 50% or 60% lecithin, 10% cholesterol, and 20% dicetyl phosphate. Two batches of liposomes were used in these studies. These preparations, calculated to contain 0.29 μM/ml and 2.27 μM/ml, respectively, of gp were analyzed spectrophotometrically to determine whether they display the characteristic absorbance of gp. Sperm exposed to gp-liposomes were examined under the fluorescent microscope for evidence of incorporation of gp.

Arbacia sperm exposed to free gp at a concentration of 25 μM/ml for 10 min stop moving and fluoresce along the entire cell surface but with greatest intensity at the mid piece. After a similar period of exposure, evidence of sperm fluorescence is minimal when gp-liposomes are added, and 80% of the sperm retain motility. After 30 min, however, the fluorescence of sperm so treated becomes evident, and motility has ceased. Similarly, when the end point used is the ability of sperm to raise the fertilization membrane of *Arbacia* eggs, the inhibitory action of gp is delayed by incorporation within liposomes. These observations demonstrate that biological effects of gp, *i.e.*, inhibition of motility and fertilizing capacity of sperm, can be elicited by delivering the drug in the form of a constituent of the lipid phase of liposome membranes.

Melittin-induced germinal vesicle breakdown in nonclidoic oocytes. Amrut K. Deshpande and S. S. Koide, The Population Council, NY.

The bee venom, melittin, is an amphipathic polypeptide with a known sequence of 26 amino acids. It consists of a hydrophobic and a basic hydrophilic segment, possesses lipolytic activity and stimulates Na-K pump activity. Melittin at 1.5 μM induces germinal vesicle breakdown (GVBD) in 98% of Stage VI (Dumont) oocytes and in 96% of Stage IV oocytes of *Xenopus laevis.* Progesterone (30 μM) induces GVBD in 100% of Stage VI oocytes. However, the steroid was ineffective in inducing GVBD in Stage IV oocytes. The onset of GVBD occurs earlier (shorter lag period) with melittin than with progesterone, *i.e.*, 3 h compared to 5 h. Melittin induces an unusual morphological mottling of the animal pole. The occurrence of mottling appears unrelated to GVBD.

When Stage VI oocytes are microinjected with 60 nl of 3 mM melittin solution, only 48% showed GVBD. Moreover, when isolated germinal vesicles are incubated in modified Barth's medium containing 4 mM melittin, they remained intact. Portions of cytosol obtained from melittin-treated oocytes and microinjected into Stage VI oocytes induce GVBD in 60% of the oocytes. The present findings suggest that melittin acts by interacting with the plasma membrane rather than directly on the germinal vesicle and acts by inducing or activating a maturation promoting factor.

Melittin at varying concentrations does not induce GVBD in *Spisula* or *Chaetopterus* oocytes. These invertebrate oocytes show lytic changes when incubated in sea water containing melittin at concentrations greater than 6 mM.

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The effects of chlorpromazine and quercetin on egg maturation and activation in Chaetopterus pergamentaceus. William R. Eckberg (Department of Zoology, Howard University, Washington, D. C.) and Alan G. Carroll.

Ca^{2+} ions seem to be universal regulators of egg maturation and activation. Accordingly, we have used inhibitors of various aspects of Ca^{2+} metabolism to determine the effects on germinal vesicle breakdown (GVBD) and egg activation. Ionophore A23187 induced GVBD and fertilization envelope elevation over a wide range of concentrations and in the absence of external Ca^{2+}. High concentrations (30–100 μg/ml) of ionophore induced differentiation without cleavage. Quercetin, a potent inhibitor of Ca^{2+} pumping by the sarcoplasmic reticulum, induced GVBD but not egg activation, with a half-maximum effect at 10–12 μM. This effect was independent of external Ca^{2+}. Chlorpromazine blocked GVBD in natural seawater and 60mM K^+ artificial seawater, with a half-maximum effect at 1.5–2 μM. Chlorpromazine inhibited purified *Chaetopterus* calmodulin activity *in vitro* with a half-maximum effect at 40–60 μM. To test whether a light-induced free-radical form of chlorpromazine was responsible for the concentration difference, we repeated the experiments in the dark. In these experiments, chlorpromazine inhibited GVBD with a half-maximum effect at 50–100 μM. Chlorpromazine activated eggs at 40–60 μM in light or darkness.

The results of these studies indicated (1) that Ca^{2+} fluxes regulate GVBD ahd egg activation, (2) that these fluxes can be internally generated, (3) that prevention of Ca^{2+} pumping (sequestration) can induce GVBD but not egg activation, (4) that calmodulin and probably another chlorpromazine-sensitive molecule or event are involved in triggering GVBD, and (5) that calmodulin may be a negative effector of egg activation. These results show the importance of multiple independent Ca^{2+}-regulated pathways in the control of egg maturation and activation.

The source and behavior of the pyridine nucleotide fluorescence in single, marine, invertebrate eggs. ANDREW EISEN (Univerity of Pennsylvania) AND GEORGE T. REYNOLDS.

Changes in the levels of the reduced pyridine nucleotides NAD(P)H at fertilization have been measured in six species of marine eggs. We used a fluorescence microscope with (1) a sensitive photomultiplier and (2) an image intensifier-video system. We find that the 365-nm-excited 454-nm-autofluorescence of the egg is photosensitive; under high illumination, 50% of the fluorescence is bleached within a few seconds. The fluorescence approximately doubles from this lower level at fertilization. Under low illumination, bleaching can be reduced to less than 10% for a 5–10 min exposure. The change at fertilization then is species dependent: less than 10% increase in *Arbacia punctulata, Lytechinus variegatus, Spisula solidissima,* and *Chaetopterus pergamentaceus*; greater than 50–150% in *Strongylocentrotus purpuratus* and *Lytechinus pictus*. The fluorescence always increases over about 2 min after fertilization and, under low illumination, remains stably elevated for at least 30 min. The fluorescence is distributed uniformly throughout the unfertilized egg; it bleaches uniformly, and it increases uniformly at fertilization. With centrifugally stratified eggs (particularly *A. punctulata*), we find the fluorescence before and after fertilization is associated with the known distribution of mitochondria. Stratified eggs stained with the mitochondrion-specific dye, Rodamine 123, reveal a distribution of mitochondria identical to that of the 365-nm-excited autofluorescence. That the 365-nm-excited autofluorescence is due to the reduced pyridine nucleotides is suggested by the fluorescence emission spectra obtained from single eggs or from the mitochondrial band of single stratified eggs. The egg spectra, which do not change upon fertilization, are similar to a spectrum of a reference solution of NADH. The association of the change in the NAD(P)H fluorescence with the mitochondria at fertilization is surprising, considering the generally accepted involvement of NAD kinase and NADPH in cytoplasmic, rather than mitochondrial, metabolism.

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External calcium requirements for oocyte maturation in the surf clam Spisula solidissima. PIERRE GUERRIER, FRANCOIS DUBÉ, AND MARC MOREAU (Station Biologique, 29211 Roscoff, France).

Activation of the bivalve oocyte triggers germinal vesicle breakdown (GVBD) and maturation divisions in the absence of exocytosis of the cortical granules. Using *Barnea candida* oocytes, we found previously that fertilization or artificial activation induced by an excess of KCl required the presence of external calcium for about 3 min and involved a ^{45}Ca influx, whose inhibition by D600 precluded the biological response. By contrast NH_4Cl activation was found to occur in the complete absence of external calcium, which contrasted with a previous report concerning *Spisula* oocytes (Finkel and Wolf 1980, *Gam. Res.* **3**: 299–304). The present data obtained from *Spisula* confirm our conclusions and suggest that calcium and pH changes are both necessary for triggering activation since:

1. Excess KCl (Threshold 15–28 mM) induces GVBD in less than 12 min at 16°C, provided that at least 1 mM Na^+ and 0.9 mM Ca^{2+} are present. Lower calcium concentrations (0.3–0.6 mM) delayed GVBD. This KCl treatment (usually 53 mM) proved to be effective even when it triggered a lower H^+ efflux than did the sperm (0.42 to 0.58 instead of 0.91 picomoles H^+/oocyte). We found, however, that the stepwise addition of below-threshold amounts of KCl, which produced a total H^+ release similar to that obtained with sperm, could not trigger activation. Similarly, adding 1 mM Na^+ alone to Ca^{2+}-, Na^+-free, KCl-supplemented seawater induced a significant H^+ release (0.48 picomoles H^+/oocyte) that

did not result in activation unless Ca^{2+} was added. These results along with the fact that calcium permeability blockers such as D600 (75–300 μM), Co^{2+} or Mn^{2+} ions (10 mM) inhibit the biological response, seem to confirm that a KCl-dependent calcium influx is required in these conditions, and not only a simple change in the internal pH.

2. The ionophore A23187, which exchanges Ca^{2+} for H^+ to the same extent as does sperm, also proved effective only in the presence of external calcium, with or without Na^+. Such an effect was found to be insensitive to D600 (up to 750 μM) but inhibited by Mn^{2+} and Co^{2+} which may compete with Ca^{2+}.

3. Oocytes were readily activated by ammonia (NH_4OH-titrated seawater or 10–40 mM NH_4Cl, pH 9) in calcium-free seawater containing 2 mM EGTA, even when 10 mM Mn^{2+} or Co^{2+} are present. The fact that this effect is blocked by D600 (300 μM) suggests, however, that ammonia could eventually release intracellular Ca^{2+}, since this drug is also known to block the 1-methylade-nine calcium release in the starfish oocyte.

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Phosphorylation of the 40S ribosomal protein rp31 in Arbacia *eggs at fertilization requires both Ca^{++} and an increase in intracellular pH.* TIM HUNT AND DENNIS BALLINGER. Department of Biochemistry, University of Cambridge, England.

A 40S ribosomal protein, M_r 31,000 (rp31) becomes phosphorylated shortly after fertilization of *Arbacia punctulata* eggs. A specific phosphatase, which dephosphorylates this protein, is present in extracts of unfertilized eggs but absent from fertilized egg extracts (Ballinger and Hunt, *Dev. Biol.*, in press). This summer we examined the effects of the artificial activating agents, the calcium ionophore A23187, and NH_4Cl on the phosphorylation of rp31. Addition of sperm or A23187 activates protein synthesis with identical kinetics and causes the phosphorylation of rp31 to increase, starting at the same time as protein synthesis takes off, and continuing to increase for about 15–20 min. By contrast, 10mM NH_4Cl activates both protein synthesis and ribosome phosphorylation only about half as well. In choline-substituted Na^+-free seawater, 20 μM A23187 raises the fertilization membrane of the eggs, but neither activates protein synthesis nor stimulates phosphorylation of rp31. The presence of 40 mM NaCl in this artificial seawater allows both processes to occur normally.

These data suggest that the phosphorylation of rp31 requires both an intracellular calcium transient and an increase in intracellular pH, although there may be a small Ca^{++} transient when eggs are exposed to NH_4Cl. Assay of the phosphatase in extracts of eggs activated by A23187 or NH_4Cl showed that the phosphatase is lost with A23187 in normal seawater but not in Na-free seawater; that it does not disappear from ammonium-activated eggs; and that it is completely lost by 20 min after fertilization, and considerably diminished 5 min after addition of sperm. There is thus an excellent correlation between the degree of activation of protein synthesis and the phosphorylation of rp31, and between this phosphorylation and the loss of the phosphatase. Whether the phosphorylation is a cause or a consequence of the increase in protein synthesis, however, is impossible to tell from these data.

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Peroxidase release following fertilization of Spisula solidissima. C. RICHARD LYTTLE (University of Pennsylvania) AND SUSANNA AMELAR.

The release of a peroxidase activity has been shown to occur seconds after fertilization in the sea urchin (*Strongylocentrotus purpuraus*) and has been proposed to play a role in the formation of the fertilization membrane. In these experiments, we examined the *Spisula* oocytes for peroxidase activity and its release following activation both by insemination and application of chemicals. Peroxidase activity was extracted by lysing the oocyte in 10 mM Tris-HCl pH 7.5 buffer (1 ml packed cells per 10 ml buffer). The lysate was centrifuged at 20,000 × g for 30 min at 4°C and the resulting supernatant was used to characterize the enzyme. The peroxidase from the *Spisula* oocytes exhibited some properties common to peroxidases: it was inhibited by KCN and NaN_3 and had a sedimentation value in the 4–5 S range. It also had several distinguishing properties namely a high pH optimum and a lack of affinity for concanavalin A. Such an affinity is usual in peroxidases from both plant cells and various mammalian cell types. In studying the release of peroxidase activity, we inseminated washed oocytes with sperm. At regular time intervals (15 min), duplicate 2 ml samples were removed, the fertilized eggs were pelleted by hand centrifugation, and the supernatant was assayed for peroxidase activity. During the first 30 min following fertilization, there was little change in activity. A slight and gradual increase was seen 30–60 min and a dramatic increase 90–120 min following fertilization. The level of activity decreased gradually between 120 and 280 min and then sharply between 280 and 400 min, when it returned to near initial levels. Similar patterns of release of peroxidase activity were seen when the oocyte was activated by KCl (70 m*M*) or the ionophore A23187 (0.4 μg/ml), except the peroxidase was released approximately 50–60 min later. The addition of 0.5 m*M* nicotinamide at fertilization had previously been shown to prevent cleavage and a higher concentration (10 m*M*) prevents germinal vesicle breakdown. In our studies, both concentrations of nicotinamide prevented the peroxidase release. Thus, we have shown that a peroxidase is present in the *Spisula* oocyte and that this enzyme is probably released during the early stages of cleavage.

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Evidence for an oocyte-agglutinating factor from Spisula *sperms.* LIN-FANG WANG AND S. S. KOIDE. Population Council, Rockefeller University, New York, N. Y., 10021.

The initial interaction of sperm with egg at the time of fertilization involves membrane-membrane fusion. The present study was undertaken to clarify the molecular mechanism of *Spisula* sperm-oocyte interaction. To attain this objective, *Spisula* oocytes were incubated in a medium containing 1 *M* urea, 5 m*M* EDTA, 10 m*M* Tris-HCl, pH 7.4, filtered seawater (FSW) at 22°C for 10 min. The treated oocytes were washed 3 times with FSW and collected by sedmintation. The induction of GVBD in the oocytes was observed 15 min after insemination. The ratio of sperm to oocyte in the assay system was 150:1. More than 95% of the control (untreated) oocytes showed GVBD, while less than 2% of the treated oocytes underwent GVBD.

Spisula sperm incubated in the same EDTA-urea medium became immobile within 30 sec. Motility was restored when sperm were washed immediately with FSW three times and collected by centrifugation. However, sperm treated for three min or longer remained immobile even after extensive washing. Addition of 2-mercaptoethanol (10 μl/ml), dithiothreitol (10 m*M*) or ATP (1 m*M*) (final concentrations) to the treatment medium did not protect nor restore motility when added after the washing.

Spisula sperm or oocytes were suspended in the above treatment medium by mixing in a glass homogenizer with five gentle strokes. The suspension was stirred for 2 h at 22°C and centrifuged at 10,000 *g* for 20 min. Supernatant was dialyzed against distilled water and the retentate lyophilized. On adding sperm extract to a suspension of oocytes, agglutination of the cells occured. However, aggregation did not take place with other combinations of *Spisula* and *Arbacia* oocytes or sperm with their extracts.

Mixing extracts of *Spisula* oocytes and sperm in varying proportions resulted in a precipitin reaction. The occurrence of turbidity was confirmed by measuring the absorbance of the mixtures at 640 nm. The present results suggest that the extracts contain membrane macromolecules involved in sperm-oocyte interaction.

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MARINE RESOURCES

Laboratory culture of Bahamian bivalve molluscs: reproduction and general biology. CARL J. BERG, JR. (Marine Biological Laboratory) AND PHILIP ALATALO.

Field data and laboratory studies are being coordinated to study the biology of tropical bivalve molluscs, particularly Bahamian clams with potential commercial importance. Monthly field sampling on Grand Bahama Island and surrounding areas has revealed dense assemblages of the Sanguinolarid clam, *Asaphis deflorata* (Linné) among beds of *Thalassia.* The Lucine clams *Codakia orbicularis* (Linné) and *Lucina pensylvanica* (Linné) are large clams capable of inhabiting hydrogen sulfide-rich sediments 4–12 cm below the surface. Histological analysis of preserved samples indicates gradual ripening of the gonads through spring in *A. deflorata, C. orbicularis,* and *Tellina listeri* Röding. Planktotrophic larval development can be inferred from egg sizes in the following clams: *A. deflorata*, 60 μm; *T. listeri*, 121 μm; *C. orbicularis*, 130 μm; and *L. pensylvanica*, 134 μm.

Adult and juvenile clams transported to Woods Hole, MA, are kept in a heated flow-through seawater system at temperatures simulating those of Grand Bahama Island (22°–32°C). Despite supplemental feeding of tropical species of phytoplankton, no significant change in body length, height, depth, and wet weight occurrred in most clams over 5 months. While somatic growth appeared negligible, gonad development occurred in *A. deflorata* and *C. orbicularis* held in the laboratory. Attempts to induce spawning were unsuccessful from February until early August. In mid-August, *A. deflorata* was stimulated to spawn in response to thermal shock, salinity shock, and treatment with a basic solution of 5 m*M* hydrogen peroxide. In 7–12 h at 27°C fertilized eggs develop into trocophore larvae 64.8 ±

5.5 μm in diameter. Straight-hinge veligers develop 23 h following fertilization, and are approximately 74 × 90 μm. Larvae cultured at salinities of 36‰, 34‰, and 32‰ and temperatures of 27°C and 25°C display similar growth rates in all salinities except for irregular increases at 27°C. The average daily increase is 5 μm in shell length. On day 7, veliger length ranged from 114–123 μm.

The authors gratefully acknowledge the support of this research through a grant by the Wallace Groves Aquaculture Foundation, Freeport, Bahamas.

Laboratory culture of Agardhiella subulata *(Rhodophyta), Strain A_1.* THOMAS R. CAPO (Marine Biological Laboratory).

The successful laboratory culture of many biomedically important marine invertebrates depends on consistently providing the appropriate food source for the various stages of the organisms' life cycle. Two years ago we reported the successful isolation and use of *Agardhiella subulata*, Strain A_1, as a metamorphic substrate and juvenile food source for *Aplysia californica* (Capo *et al.*, 1979, *Biol. Bull.* **157**: 360).

Various aspects of *A. subulata* culture have been studied to consistently produce algae on a year-round basis. Two-liter flasks were incubated at 21–24°C. Weekly, the algae were rinsed, weighed, and transferred to fresh medium. The culture medium is important in controlling growth rate and yield of *A. subulata* in batch culture. Algae (0.20 g wet wt) maintained in basal media (0.45 μm filtered Guillard's f/10 medium) increased by 20.4 g while algae (0.20 g wet wt) incubated in 0.45 μm filtered seawater increased by only 0.03 g after 28 days. In addition, preliminary experiments indicate that algal yields can be further enhanced by enriching the basal medium with 0.02–0.28 m*M* ammonium chloride.

Aeration proved to be almost as significant as the medium in controlling algal yields. Culture vessels, with an initial density of 0.116 ± 0.018 g algae, aerated from 0.5 to 2.0 liters/min. for 28 days, produced an average yield of 18.9 g wet weight while non-aerated vessels produced only 3.6 g wet weight.

Finally, we studied the effects of initial inoculum size and density on cumulative and mean wet weights. Initial densities of 5, 10, 20, 30, and 40 one-cm pieces of strain A_1 (0.011 ± 0.003 g), with all apices removed, yielded similar cumulative wet weights after 35 days: 21.1 g ± 5.3 g. A starting density of 1 piece (0.012 ± 0.002 g), however, produced a cumulative wet weight of 8.4 ± 4.0 g for the same 35-day interval. Hence, the mean wet weight per piece and specific growth rate were inversely proportional to the initial density. Cultures inoculated with 1 one-cm piece of *Agardhiella* averaged 8.4 ± 4.0 g, while cultures inoculated with 40 one-cm pieces averaged significantly less: 0.5 ± 0.2 g per piece. The mean instantaneous growth rates (k) were 0.187 and 0.109, respectively.

With the techniques for *Agardhiella* culture described here, we can now investigate factors affecting metamorphosis, growth, and nutrition of juvenile *Aplysia californica.*

This work was supported by grant GM23540-06 from the National Institute of General Medical Science.

Observations of prey preference and predatory behavior in Busycon carica *(Gmelin) and* B. canaliculata *(Linn.).* JONATHAN P. DAVIS (Yale School of Forestry and Environmental Studies).

Coexistence between a pair of sympatric, predatory, marine prosobranch gastropods of the genus *Busycon* may be maintained by differences in diet between the species *Busycon carica* (Gmelin) and *B. canaliculata* (Linn.) in coastal Cape Cod waters. This differentiation may not be based on active selection by each species, since studies assessing prey selection on the basis of chemoreception indicate that adults of both species prefer the same range of prey (which includes *Ensis directus* (Conrad), *Aequipecten irradians* (Lam.), *Spisula solidissima* (Dill.), and *Mya arenaria* Linn.). Characteristics of this group include an inability to maintain valve closure for long periods, and an active escape response when touched by foraging predators.

Preliminary laboratory prey-preference studies with juvenile and adult *Busycon* suggest that *B. canaliculata* is more efficient than *B. carica* in capturing and processing these mobile bivalves. Though *B. carica* prefers bivalves from this prey spectrum, it is more often observed eating sedentary bivalves such as *Mercenaria mercenaria, Mytilus edulis* Linn., and *Crassostrea virginica* (Gmelin). A study of foraging behavior indicates that *B. canaliculata* is significantly faster moving than *B. carica* in both short-term (30 sec), and long-term (more than 4 min) test periods.

The above hypothesis is supported by field observations of *Busycon* species ratios in a variety of habitats, and the available prey spectrum in Cotuit Bay, Massachusetts. These observations support the hypothesis that the distribution of bivalve prey may contribute to the relative densities of *Busycon* species in the near-shore marine environment. This may be due to different abilities to capture prey.

I acknowledge the invaluable support of the Marine Biological Laboratory, and the laboratory of

Carl J. Berg, Jr. Financial support for this work was provided by the Yale School of Forestry and Environmental Studies, and the Richard King Mellon Foundation.

*Diagnostic findings of the Laboratory for Marine Animal Medicine at the Marine Biological Laboratory** during the summer of 1981.* LOUIS LEIBOVITZ* AND JOHN VALOIS** (New York State College of Veterinary Medicine, Cornell University,* Ithaca, New York 14853).

The incidence, etiology, and course of observed diseases of marine animals maintained at the Marine Resource Center of the Marine Biological Laboratory and in the laboratories of the scientific community of Woods Hole is presented. The following percent daily mortality was observed in captive marine animals during a 60-day summer period at the MBL: finfish—*Paralichthys dentatus* 15.8, *Raja erinacea* 9.0, *Squalus acanthias* 7.6, *Opsanus tau* 2.3; echinoderms—*Arbacia punctula* 16.0, *Strongylocentrotus purpuratus* 7.2, *S. drobachiensis* 3.6, *Echinarachnius parma* 0.5; arthropods—*Limulus polyphemus* 3.2; molluscs—*Loligo pealei* 13.4, *Placopecten magellanicus* 2.3, *Spisula solidissima* 0.08; and annelid—*Glycera* sp. 22.1. Newly reported diseases of *Arbacia, Strongylocentrotus, Echinarachnius,* and *Daphnia* are described. The relationship of disease to management and holding facilities is discussed. Further work is in progress to further describe the diseases of captive marine animals.

The Laboratory for Marine Animal Health is a cooperative effort of the School of Veterinary Medicine at the University of Pennsylvania, the New York State College of Veterinary Medicine at Cornell, and the MBL. It is funded in part by the National Institute of Health.

Structural studies on aplysioviolin. ROBERT R. TROXLER, GWYNNETH D. OFFNER AND THOMAS R. CAPO (Marine Biological Laboratory).

The opisthobranch mollusc, *Aplysia californica*, releases a purple fluid from the ink gland (Blockmann's gland) following mechanical stimulation. Rudiger (Hoppe-Seyler's *Z. Physiol. Chem.* **348**, 120, 1967) named the purple pigment "aplysioviolin" and suggested that it was structurally equivalent to the monomethyl ester of phycoerythrobilin, which is the prosthethic group of phycoerythrin contained in red algae eaten by *Aplysia* species. Since little is known about the composition of *A. californica* ink, the present work was undertaken to determine the pigment, peptide, and protein composition of this biological fluid.

Ink collected from animals was freeze dried and stored at −20°C until used. Assay of lyophilized ink by the method of Lowry indicated that 35% of the dry weight was peptide or protein. Amino acid analysis before and after hydrolysis (6 *N* HCl, 110°C, 22 h) showed that unhydrolyzed samples contained free serine, proline, and histidine in amounts equivalent to that in hydrolyzed samples, whereas the latter contained 19 amino acids (cysteine most abundant by a factor of 3) plus amino sugars.

Lyophilized ink taken up in 15% acetic acid was chromatographed on a Sephadex G-25 column equilibrated in the same solvent. Assay of eluate at 230, 280, and 550 nm showed that higher molecular weight protein (A_{230}) was absent and purple pigment eluted in the same volume as ^{14}C-proline standard. This indicates that aplysioviolin occurs in ink in a low molecular weight form (not covalently attached to protein). All fractions of eluate containing purple pigment contained cysteine that could not be carboxymethylated with iodoacetamide, suggesting that cysteine was linked to aplysioviolin. The molar ratio of aplysioviolin to cysteine was 9:1 (all cysteine bound to pigment but 90% aplysioviolin as free pigment).

The absorption spectrum of ink in water, methanol-5% HCl, 30% acetic acid, and chloroform displayed a single major band at 550, 585, 585, and 532 nm, respectively, indicating that aplysioviolin is the primary pigment in ink and that other pigments (phycocyanobilin, urobilinoids) are not quantitatively significant.

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NEUROBIOLOGY

Vision in Lumulus *mating behavior.* ROBERT B. BARLOW JR. (Syracuse University), LEONARD C. IRELAND, AND LEONARD KASS

Although the *Limulus* visual system has been studied extensively for more than 50 years, the role of vision in the animal's behavior is not known. We investigated the problem by studying *Limulus* mating behavior, which is the only well known behavior the animals exhibit in their natural habitat. Along the Eastern coast of the North Atlantic *Limuli* move in from deep water, pair off, and build nests near the water's edge at high tide. We observed the activity of males in the vicinity of cement castings placed 4 m below the high water line on the South side of Mashnee Dike, Cape Cod, MA. Cement castings were made of an adult female carapace (27 cm width), a hemisphere (29.5 cm diameter), and a cube (16.5 cm/edge), with the exposed surface area of the hemisphere and cube equated to that of the female carapace (1365 sq cm). Each casting was painted either black, grey, or white.

Male *Limuli* are attracted to the cement castings. The degree of attraction depends on both the form and the shade of the castings. We determined the degree of attraction by counting the number of males in contact with the castings during eight high tides, four at night and four during the day. Total number of contacts counted was 6988. The number of males was highest around the female models and lowest around the cubes. Thus *Limulus* can discriminate form. When the shade of the castings is taken into account, black castings yielded the highest percentage (40%) of male contacts both during the day and at night. The attraction to grey and white depended on time of day. Combining the data on both form and shade showed that black *Limulus* was the most attractive casting and white cubes the least.

We conclude that *Limulus* can discriminate both form and shade during mating behavior. The discrimination of form may result from information transmitted by several sensory systems. The discrimination of shade requires vision.

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cAMP and adenylate cyclase probably do not mediate excitation in Limulus *ventral photoreceptors.* J. E. Brown, U. B. Kaupp, and C. C. Malbon (S.U.N.Y. Stony Brook).

Clusters of dark-adapted photoreceptor cells from ventral eyes of *Limulus* were homogenized in 250 m*M* K-phosphate buffer (pH 7.0) and assayed for adenylate cyclase activity. Aliquots of homogenates (containing 4–6 μg protein) were incubated at 30°C for 30 min in 0.2 ml containing 25 m*M* Tris·HCl (pH 7.5), 5 m*M* $MgCl_2$, 20 m*M* creatine phosphate, 100 units/ml creatine phosphokinase, 0.5 mg/ml bovine serum albumin, 1 m*M* cAMP, 0.1 m*M* ascorbic acid, and 0.25 m*M* [$\alpha^{32}P$] ATP (250 cpm/pmol). cAMP was chromatographically isolated and assayed according to Salomon *et al.* (1974, *Analyt. Biochem.* **54**: 541–548). Basal adenylate cyclase activity was 10–20 $pmol^{-1} \cdot mg\ protein^{-1} \cdot 30$ min. Adenylate cyclase activity was increased by additions of: 10 m*M* NaF, two- to tenfold; 0.1 m*M* GTPγS, fourfold; 0.1 m*M* Gpp(NH)p, threefold; 0.1 m*M* forskolin, fourfold; and 10 μg activated cholera toxin, tenfold. Clusters of ventral eye photoreceptors were also assayed for cAMP content by radioimmunoassay (NEN kit) after homogenization in 0.1 *N* HCl. Homogenates were boiled (3 min) and centrifuged; the lyophylized supernatant was assayed for cAMP and the pellet was assayed for protein. For dark-adapted eyes, there was about 15 pmoles cAMP/mg protein. Bathing eyes in seawater containing 0.1 m*M* forskolin increased the cAMP content about tenfold; bathing in 10 m*M* NaF (pH 7.0) did not change the cAMP content. Previous electrophysiological experiments showed that injecting GTPγS or Gpp(NH)p intracellularly or bathing eyes in seawater containing 10 m*M* NaF (pH 7.0) induced "quantum bumps" in the dark. Similar quantum bumps are normally induced by the absorption of single photons and are evidence for excitation of the photoreceptor. Bathing eyes in 0.1 m*M* forskolin or injecting activated cholera toxin intracellularly did not induce quantum bumps in the dark. Therefore procedures that increase the activity of adenylate cyclase or the cAMP/mg protein in ventral eyes are uncorrelated with induction of quantum bumps. Thus, it is unlikely that an increase in either intracellular cAMP or the activity of adenylate cyclase mediates excitation in *Limulus* ventral photoreceptors.

Chemosensory social behavior in catfish: responses in a social context. Bruce Bryant (Boston University Marine Program).

Behavioral processes such as recognition of gender, social status, or familiarity may be as important in the social behavior of fish as pheromone-mediated attraction. While stereotyped attraction occurs in socially isolated fish, the full expression of more complex chemosensory social behavior requires the presentation of stimuli in a more natural social context. Brown bullhead catfish (*Ictalurus nebulosus*), a fish that exhibits territorial/dominance behavior even outside of the reproductive season, was studied to determine the importance and possible functional significance of conspecific chemical stimuli in the fish's social behavior.

Heterosexual, non-breeding pairs of fish were observed before and during the presentation of chemical stimuli (tank water containing body odor) from male and female conspecifics. A repertoire of 50 stereotyped behavior units was used to quantify responses of fish. Control observation periods of 15 min were followed by experimental periods of 15 min, during which background siphon flow was changed to stimulus flow. Stimuli were presented as slow flows into unoccupied shelters in aquaria.

During presentation of body odor stimuli, fish were attracted to the site of introduction. Significant increases in a number of behavioral units were observed. The general activity of the fish increased from control to experimental periods, as did several behaviors directed at the stimulus introduction tube ($p < .05$), Wilcoxon. In addition, "surface nose," a non-social behavior, decreased during stimulation. Significant increases ($p < .05$) in "scratch," a possible substrate marking behavior and "mouth display" were observed only in males. "Lateral display," a less intense aggressive display, increased in both sexes.

Attraction to the stimulus and increases in general activity suggest that the fish were responding to novelty or change in the chemical environment. Attention to the stimulus changed during the experimental period to aggressive behavior directed at the other fish. This indicated that chemical stimuli must be supplemented with other sensory cues to elicit directed aggressive behavior. Sexual dimorphism in the response to odor stimulation is consonant with the males' role in territorial dominance hierarchies.

The effect of internal sodium ions on the action potential and reversal potential in squid axon. DONALD C. CHANG (Baylor Coll. of Med.).

In the ionic theory of membrane potential, the overshoot of the action potential is basically the Nernst potential of Na^+ (V_{Na}) with a slight mixture of the K^+ Nernst potential. In principle, the action potential cannot exceed the sodium potential. Tasaki and Luxoro (1964, *Science* **145:** 1313–1315) reported earlier an observation that as the internal Na^+ concentration increases, the action potential is higher than that of V_{Na}. This finding was later dismissed by Chandler and Hodgkin (Chandler and Hodgkin, 1965, *J. Physiol.* **181:** 594–611) as an artifact caused by capacitative coupling between the internal electrode and the perfusion cannula. Because of the fundamental importance of this problem, we decided to reinvestigate the effect of internal Na^+ ions on the action potential. Using an experimental design which avoids the capacitative coupling between electrode and cannula, we studied the action potential of squid axon at various internal sodium concentrations. Furthermore, we employed the voltage-clamp technique to measure the reversal potential (V_{rev}), which is theoretically equal to the action potential but has the advantage of being unaffected by leakage current.

In studying over 25 axons with F^- as the major anion, we found that the V_{rev} is smaller than V_{Na} when $[Na]_i$ is less than 50 m*M*. However, V_{rev} becomes larger than V_{Na} when $[Na]_i$ is equal to or more than 100 m*M*. For example, the V_{rev} measured at $[Na]_i$ = 400 m*M*, averaged over 9 axons, is 10.7 ± 1.5 mv, while the V_{Na} calculated based on the Na^+ activity ratio is 4.5 mv. The action potential also is found to be higher than V_{Na} at higher Na^+ concentrations (200 m*M* or over). The average action potential from four healthy axons is 9.4 ± 1.0 mv, which exceeds V_{Na} by about 5 mv. The cause of this internal Na^+ effect is being investigated.

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Low-angle X-ray diffraction patterns of fish retinas. A. R. CZETO, A. R. WORTHINGTON, AND C. R. WORTHINGTON (Carnegie-Mellon University).

We have investigated the molecular structure of the retinal photoreceptors of fish by low-angle X-ray diffraction. It is known from previous X-ray studies on vertebrate retina that diffraction has been recorded from rod cells but not from cones. Well defined X-ray patterns have been reported for a number of vertebrate retinas: frog, cattle, rat, monkey, toad, salamander, and mudpuppy. The present study on fish retinas was designed to answer two immediate questions: (1) can diffraction be recorded from rod and cone cells; and (2) what is the structure of visual cells of fish?

We report low-angle X-ray patterns from rod outer segments of fish: marine teleosts (toadfish, sculpin, and porgy), a marine elasmobranch (skate), and a freshwater teleost (carp). The retinas were dissected free from the pigment epithelium in Ringer's solution. One-mm strips of bleached retina then were mounted in a specimen chamber with excess Ringer's solution. The Ringer's solutions were either marine or freshwater teleost solutions or skate Ringer's solution. X-ray exposure times were usually 1 or 2 h, using slit collimation.

No diffraction was recorded from cones. Our X-ray diffraction patterns from fish retinas come from rod cells. The first eight orders of diffraction of d, the disc-to-disc repeat distance of the rod outer segments, were obtained. The various d-values were: toadfish, 310 Å; sculpin and porgy, 290 Å; skate, 330 Å; and carp, 275 Å. The intensity variation of the X-ray patterns from fish was similar to the previous X-ray patterns of rod outer segments from frog and the other vertebrates listed above. The phases for the frog reflections have been uniquely determined in earlier work. Using these phases, Fourier profiles of the disc structure of the five varieties of fish have been obtained.

This work was supported by NIH grant NS 09329.

Overlap of interganglionic dendritic fields of mechanosensory cells in leech segmental ganglia. SUSAN A. DERIEMER, EDUARDO R. MACAGNO (Dept. of Biological Sciences, Columbia University), AND KENNETH J. MULLER.

One way to study the accuracy of regeneration of a cell into a region it previously occupied in the central nervous system is to determine the position of its regenerated processes with respect to those of local cells. This can best be done by marking the regenerated cell with an intracellular dye and a local cell with a different dye (Macagno *et al.* 1981, *Brain Res.* **217**: 143–149). In previous studies (DeRiemer and Macagno, 1981, *J. Neurosci.* **1**(6): 650–657), we found that within the segmental ganglia of the medicinal leech, the branching pattern of one of the mechanosensory cells that respond to touch (T cells) could serve as such a marker for the branching pattern of another T cell. The experiments reported here extended our findings to the relationships between T cells from adjacent ganglia. Cells were injected with horseradish peroxidase, which was allowed to diffuse for 2–4 days by maintaining the ganglia in L15 culture medium at 14°C. A second cell in the adjacent ganglion was then filled with Lucifer Yellow and the tissue processed by the method of Macagno *et al., ibid.* Consistent with our previous observations, the dendritic fields of T cells in adjacent ganglia overlap markedly with those of local T cells. Thus, a local T cell can serve as a positional marker for the regeneration of processes from a T cell in an adjacent ganglion.

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A calibrated system for the generation and detection of near-field fluid displacements. T. A. DOURDEVILLE, E. W. HEINEKE, AND AD. J. KALMIJN (W.H.O.I).

The hydrodynamic events attending the motion of an immersed body comprise a "near-field" non-propagated incompressible flow and a "far-field" propagated compressional wave. Detectors of periodic and aperiodic near-field water displacements exist in the lateralis system of aquatic vertebrates and in various mechanoreceptors of invertebrates. The biological significance of low-frequency near-field stimuli has, however, remained in controversy due to a lack of instrumentation capable of measuring and simulating naturally occurring displacement fields.

Dipole displacement fields were produced by feedback-controlled excursions of a rigid syntactic-foam sphere of 1.25-cm radius. The system was designed to yield excitation amplitudes up to 1 mm over the frequency range from dc to 100 Hz. The near-field displacements generated may be described by a potential field for all but the lowest frequencies, where the water viscosity complicates the physical picture. The chamber enclosing the test space offered well defined, stiff boundaries, eliminating any free surfaces. The boundary effects were accounted for numerically by a computer program based upon the theory of images.

Fields generated by natural sources or by the excitation sphere were recorded with a second 1.25-cm-radius, syntactic-foam sphere, suspended by a slender graphite-fiber truss with bridle. By choosing a neutrally bouyant sphere and adjusting the stiffness of the suspension bridle, the system could be tuned to achieve an output amplitude flat within 3 db from 1 to 100 Hz, allowing reliable measurements of submicrometer fluid displacements. The performance of the field generation and recording system closely matched theoretical expectations.

In preliminary experiments, the fish *Gymnotus carapo* executed obvious feeding responses with respect to periodic water displacements in the range from about 1–5 Hz, while the copepod *Centropages typicus* escaped radially away from a one-cycle sinewave stimulus, $^1/_{30}$ sec in duration. These observations exemplify the importance of near-field displacement detection in the sensory repertoire of aquatic animals.

This work was conducted under contract with the Office of Naval Research, N00014-79-C-0071, Ad. J. Kalmijn, Principal Investigator. T. A. Dourdeville and E. W. Heineke, W.H.O.I., participated in partial fulfillment of their graduate studies.

A quantitative study of the branching pattern of T cell processes in Hirudo medicinalis. DOUGLAS L. FALLS AND EDUARDO R. MACAGNO (Columbia University).

Each segmental ganglion of the medicinal leech contains six mechanoreceptive cells sensitive to light touch, the T cells. When dye injected, these cells are easily distinguished from other leech neurons by the general pattern formed by their processes. However, the location of branch points along the main process and the lengths of branches vary greatly. Synapses occur predominantly on the branches and only infrequently along the main process. Despite the variability in branching pattern, physiological studies have shown the coupling of touch cells with other identified neurons to be quite reproducible from individual to individual. Nicholls and Blackshaw (unpublished observations) found that the skin branches of a given T cell innervate non-overlapping regions of skin. This caused us to wonder if a similar exclusion principle might operate centrally.

We injected T cells with HRP and prepared ganglia as whole mounts. Using a computer-coupled microscope, we measured the distance between branch points and the length of each branch. For three ganglion- #10 T cells from two animals, each innervating ventral skin, we found inter-branch point distance along the main process to vary from 1–35 μ with no strong mode; however, we found a quite constant total number of branches (41, 41, 42). In these same cells, the lengths of individual branches, differed considerably, but the sums of branch lengths were similar for the three (1275, 1344, and 1459 μ). This suggests to us that a mechanism in development closely controlling branch number and total branch length may account in part for the specific functional characteristics of the T cells.

This work conducted under the auspices of the Neural Systems and Behavior Post-course Research Session.

Fish that taste with their feet: spinal sensory pathways in the sea robin, Prionotus carolinus. THOMAS E. FINGER (Univ. Colorado Medical School).

Sea robins (*Prionotus*) have three pairs of mobile fin rays unattatched to the pectoral fin. These fin rays are chemosensory (Bardach and Case, *Copeia* **1965:** 194–206), but have no taste buds and are innervated only by spinal nerves. The rostral end of the spinal cord has three pairs of dorsal enlargements, termed accessory lobes (Herrick 1907, *J. Comp. Neurol.* **17:** 307–327).

Horseradish peroxidase (HRP) was applied to the nerve innervating each of the fin rays. Transganglionic transport of HRP was revealed by Hanker-Yates or tetramethylbenzidine reactions. The ventral fin ray, farthest from the pectoral fin, is represented only in the caudal accessory lobe, the middle fin ray in the middle lobe, and the dorsal fin ray in the rostral lobe. Nerves innervating the pectoral fin project to that portion of the spinal cord lying immediately rostral of the accessory lobes.

To determine the central-nervous-system pathways involved in the fin ray chemical sense, injections of HRP were made into the accessory lobes, cerebellum, funicular nuclei, or thalamus. Anterograde and retrograde transport of HRP were revealed by a modified Hanker-Yates method.

Two major ascending fiber systems emerge from each accessory lobe. A direct spinocerebellar pathway arises from large neurons deep in the lobes, and terminates ipsilaterally in the corpus cerebelli. A more massive ascending system from the accessory lobes runs along the dorsolateral surface of the spinal cord to end ipsilaterally in a lateral funicular nucleus located near the spinomedullary junction. This spinofunicular pathway arises mostly from small and medium-sized neurons scattered throughout the accessory lobes. The lateral funicular nucleus, in turn, projects to the nucleus preglomerulosus of the contralateral thalamus. The multisynaptic pathway from the accessory lobes to the contralateral thalamus is similar to the spinocervicothalamic system described for mammals.

Leukotriene B_4 and phosphatidic acid are calcium ionophores: studies employing arsenazo III in liposomes. JUDY FRIDOVICH, CHARLES SERHAN, EDWARD J. GOETZL, PHILIP B. DUNHAM AND GERALD WEISSMANN (New York University School of Medicine).

Neutrophils, like other secretory cells, undergo stimulus-secretion coupling when activated by surface ligands, such as chemoattractants. Activation induces turnover and oxidation of membrane lipids as well as increments in calcium fluxes. Since stimulus-secretion coupling is mimicked by exposure of cells to calcium ionophores of fungal origin (A23187, ionomycin), we sought evidence that products of the cells' own lipid metabolism might act as "endogenous" ionophores. Of over 60 phospholipids, prostanoids, or fatty acids tested, only phosphatidic acid (>10 μM) and the chemoattractant leukotriene B_4 (LTB_4, or 5(S)12(R) dihydroxy-eicosa 6, 14 *cis*, 8, 10 *trans*- tetraenoic acid) (>150 nM) translocated Ca^{++} when added to preformed liposomes. Other products of 5- or 15- lipoxygenases (e.g. 5(S)12(R) dihydroxy-eicosa, 6, 8, 10 *trans*, 14 *cis* tetraenoic acid; 5- or 15-monohydroxy and hydroperoxy eicosatetraenoic acids) were not ionophores. Ca^{++} translocation in liposomes was concordant with the capacity of native lipoxygenase products to elicit neutrophil migration. Judged by spectrophotometry of liposome-entrapped arsenazo III, Ca^{++} translocation was induced by ionomycin > A23187 > LTB_4 > phosphatidic acid. Mg^{++} was not translocated, and liposomes remained intact, as determined by chromatographic analysis (Sephadex G-50) of leaked arsenazo III. Loss of ionophoresis after $SnCl_2$ reduction of ionomycin, A23187, or LTB_4 indicated a role for oxygen functions in Ca^{++} translocation. Phosphatidic acid, preincorporated into liposomes at >0.5 mole percent, promoted Ca^{++} translocation, which was further enhanced upon addition of LTB_4. These data suggest that phosphatidic acid (generated as part of the "phosphatidyl inositol response") and LTB_4 (a product of lipoxygenase-mediated oxidation of arachidonic acid) act in concert to enhance the calcium fluxes of stimulus-secretion coupling.

Calcium induced proteolysis and suppression of electrical excitability in the squid giant axon. PAUL E. GALLANT (Laboratory of Neurolbiology, NIMH, Bethesda, MD).

The injection of high concentrations of calcium chloride into the squid giant axon activates endogenous protease(s) (Pant and Gainer 1980, *J. Neurobiol.* **11**: 1–12.) which have access to proteinaceous structure(s) vital to the maintenance of electrical excitability.

Calcium chloride was injected in 0, 10, or 100 m*M* concentrations. After dilution by the intracellular substances, the final concentrations were aproximately 0, 1, and 10 m*M*, respectively. When 0 calcium chloride (isotonic sucrose, pH 7.3 with HEPES buffer) was injected, no proteolysis (as determined by polyacrylamide gel electrophoresis of the extruded axoplasm) or loss of electrical excitability was noted. Ten m*M* injection had the same effect in four separate axons. When 100 m*M* calcium chloride was injected, however, excitability was lost within 2 min. Profound proteolysis and liquification also occurred. Most of the proteins with molecular weights above 50,000 were digested, including the 200,000 molecular weight neurofilament protein. A high molecular weight protein (250,000) not present in extruded axoplasm, appeared after proteolysis of the intact axon, suggesting that proteolysis may have induced release of a closely adherent submembranous protein.

To determine if there was a direct link between proteolysis and loss of electrical excitability induced by calcium chloride, four axons were preinjected with leupeptin, a nontoxic protease inhibitor, and then the axons were injected with 100 or 200 m*M* calcium chloride. Calcium chloride did not induce proteolysis and also did not suppress excitability in these axons. Conduction velocity was the same as in the sucrose injected axons. The deleterious actions of intracellular calcium, therefore, depend on the activation of an endogenous protease(s), which either digests or releases vital intracellular protein(s).

Comparison of phospholipid synthesis in giant axons, stellate ganglion neuronal perikarya, and optic lobe synaptosomes from the squid, L. pealii. ROBERT M. GOULD (Institute of Basic Research in Mental Retardation, Staten Island, New York), HARISH C. PANT, AND HAROLD GAINER.

We previously demonstrated that axoplasm extruded from giant axons contains enzymes for synthesizing phosphatidylinositol, phosphatidic acid, and phosphatidylinositol phosphate (Gould 1980, *Biol. Bull.* **159**: 484). In this report, we compare lipid synthetic activity in preparations that were enriched in neuronal somata (stellate ganglia) or nerve terminals (optic lobe synaptosome), with that of intact axons and extruded axoplasm. A number of labeled precursors (choline, myo-inositol, glycerol, serine, phosphate, and ATP) were individually incubated with the various preparations. Following the incubations (1–4 h at room temperature) radioactive lipids were extracted, and the products characterized by separation on thin layer chromatograms.

Each preparation (axoplasm, homogenized ganglia, and homogenized synaptosomes) formed lipid products from every precursor. With most precursors, the radioactive lipid extracts contained a single dominant product. For choline, myo-inositol, serine, and phosphate (both inorganic phosphate and ATP), the main products were phosphatidylcholine, phosphatidylinositol, phosphatidylserine, and phosphatidylinositol phosphate, respectively. With glycerol, phosphatidylinositol and phosphatidic acid were both formed, as was neutral lipid, mainly triglyceride.

Lipid synthesis of extruded axoplasm was compared with that of axoplasm from giant axons incubated *in situ*. Only low levels of lipid product (roughly 5% that of the surrounding sheath cells) were found in axoplasm under the latter conditions. Phosphatidylethanolamine was a major product recovered from both axoplasm and sheath when axons were incubated with either glycerol or phosphate.

Additional studies compared lipid biosynthesis in ganglion cell bodies and synaptosomes with homogenates of these tissues. The integrity of the cell was found to be important. With both preparations, myo-inositol was much better incorporated, while all the other substrates were more poorly incorporated into lipid when the cells were homogenized. These data suggest that intact plasmalemma may restrict myo-inositol's accessibility to the synthetic machinery. The increased synthesis of lipid from the other precursors may reflect a requirement in these multi-step incorporations of intact membrane systems.

H.C.P. acknowledges support from the Alcohol Institute of NIH, Bethesda, MD.

Field experiments on electrically evoked feeding responses in the pelagic blue shark, Prionace glauca. GAIL W. HEYER, MELANIE C. FIELDS, R. DOUGLAS FIELDS, AND AD. J. KALMIJN (Woods Hole Oceanographic Institution).

Electroreception has been shown to present an important sensory modality enabling bottom-dwelling Chondrichthyes to perceive the weak bioelectric fields of their prey. Pelagic sharks also have a well-developed electroreceptor system, comprising numerous ampullae of Lorenzini. We therefore tested the blue shark, *Prionace glauca*, on its responses to electrically simulated prey.

Observations were made at night from a Boston Whaler, in 40 m of water, 25 km south of Martha's Vineyard, Massachusetts. Electric fields were produced by a current source and two pairs of salt-bridge electrodes, each located 30 cm from a central odor source. The electrodes and odor source were suspended 5 m beneath a glass viewing well in the bottom of the fiberglass research vessel. A direct current of 8 μA was used to simulate the bioelectric fields of prey. With the dipole electrodes 5 cm apart, this current gave rise to a voltage gradient decreasing to 5 nV/cm within a radius of 24–30 cm from the field source. Ground menhaden chum was pumped through the odor port between the two electrode pairs to attract the sharks to the observation area. According to a random sequence, one dipole was activated while the other served as the control for equal trial periods.

Naive observers recorded the number of shark attacks on each electrode pair. Out of 40 actual bites on the apparatus, 2 occurred at the odor source, 7 on the unactivated dipole, and 31 on the activated dipole. These results show a highly significant preference for the current-carrying electrodes ($P \leq .001$). Thus, despite concurrent olfactory and visual cues, these open-ocean sharks will attack an electric-field source simulating prey.

This work was conducted under contract with the office of Naval Research, N00014-79-C-0071, Ad. J. Kalmijn, Principal Investigator. G. W. Heyer, School of Veterinary Medicine, Univ. of Penn., participated through the Aquavet Program. R. D. Fields is a graduate student at the Scripps Institution of Oceanography, U.C.S.D.

Morphology and physiology of vestibular afferent and efferent neurons in the toadfish, Opsanus tau. S. M. HIGHSTEIN AND R. BAKER (Marine Biological Laboratory).

The morphology and electrophysiology of vestibular primary afferents, second order neurons, and semicircular canal efferents were studied with glass microelectrodes in the toadfish. Stimulating electrodes were placed on individual semicircular canal nerves (anterior, horizontal, and posterior) and macular nerves (utricle saccule, lagena) to ortho- and antidromically identify afferents and efferents that were penetrated either peripherally or centrally. Identified canal primary afferents (*e.g.* posterior) responded with short (<0.5 ms, electrotonic) and longer latency (chemical) depolarizing potentials to stimulation of macular but not other canal nerves (*e.g.* horizontal or anterior). Macular (*e.g.* saccular) afferents responded in a similar way to other macular (*e.g.*utricle), but not to canal stimulation. Primary canal afferents were generally not spontaneously active (except horizontal canal afferents) but were exquisitely sensitive to transmembrane currents applied through the microelectrode. Injection of less than 1 nA depolarizing currents 2–3 mm from the crista always produced a train of spikes. Acute nerve lesions demonstrated that this sensitivity resides in the peripheral terminal regions of primary afferents. By contrast, a train of spikes was recorded from efferents in response to a single shock applied to their peripheral axon; however, efferents were much less sensitive than were afferents to transmembrane currents. Individual morphology of the above primary afferents and efferents following intracellular HRP demonstrated that afferents terminated extensively in the ipsilateral vestibular nuclei, with finely branched fibers exhibiting numerous "en passant" and terminal boutons. Second-order vestibular neurons in the magnocellular part of the vestibular nucleus terminated widely in the brain stem from the medulla to the midbrain, especially in the oculomotor complex. Efferent vestibular neurons were located within 200 μ of the midline in the medulla. These cells exhibited extensive dendritic trees that spanned the entire ventral lateral corner of the medulla. The above "structure-function" studies of the vestibular afferent and efferent organization will greatly facilitate the search for the elusive behavioral role of the vestibular efferent system.

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Multiple species of transfer RNA are present in squid axoplasm. N. INGOGLIA AND A. GIUDITTA (New Jersey Medical School, Newark, NJ 07103).

The majority of RNA in squid axoplasm has been shown to be 4S RNA (Lasek *et al.* 1973, *Nature* **244**: 162–165; Giuditta *et al.* 1980, *J. Neurochem.* **34**: 1757–1760) and to have the properties of transfer RNA (Black and Lasek 1977, *J. Neurobiol.* **8**: 229–237; Giuditta *et al.* 1977, *J. Neurochem.* **28**: 1393–1395). The present experiments were performed to determine whether this tRNA can accept a variety of amino acids or only a single amino acid.

In the first experiment, pooled axoplasm, extruded from giant axons, and giant fiber lobes were homogenized in 0.1 *M* Tris-HCl, pH 7.5, and incubated in the presence of ATP, $MgCl_2$, β-mercaptoethanol, and individual ^{3}H-amino acids for 30 min at 30°C. Unincubated controls were included to monitor non-specific binding. Samples were spotted on paper fiber filters and assayed for radioactivity bound to the filter following treatment with 10% TCA. Results showed binding of ^{3}H-Arg, Leu, Met, Pro, and Tyr, to the nucleic acid fraction (radioactivity lost after hot TCA treatment). Values were 2–3 times greater at 30 min than in controls in both the axoplasm and giant fiber lobe, suggesting that tRNA in axoplasm is capable of binding a variety of amino acids. Since the reactions occurred without addition of exogenous enzymes, the results also suggest that the axoplasm contains the specific aminoacyl tRNA synthetases for the amino acids tested.

To study further the nature of the tRNA in axons, suitable samples of RNA purified from axoplasm and giant fiber lobe were incubated with 50 m*M* Tris-HCl, pH 7.5, containing KCl, $MgCl_2$, ATP, CTP, β-mercaptoethanol, glycerol, single amino acids, and a mixture of tRNA synthetases, partially purified from squid optic lobes. The binding of labled amino acids to fiber filters was substantially decreased in the absence of tRNA and by as much as 30 × S when incubated with ribonuclease. Approximately 10 times as much arginine as any other amino acid tested was bound to tRNA in both giant fiber lobes and axoplasm.

These experiments indicate that axoplasm of the squid giant axon contains a variety of tRNA species as well as of the corresponding aminoacyl tRNA synthetases. In addition, on the basis of our semiquantitative analysis, the concentrations of individual tRNA species in the axoplasm and the giant fiber lobe appear to be similar.

An X-ray diffraction study of fish corneas. H. INOUYE AND C. R. WORTHINGTON (Carnegie-Mellon University).

We have studied the collagen fibrils of the cornea of two marine fishes by low-angle X-ray diffraction. Previous X-ray diffraction studies refer to beef corneas (Goodfellow *et al.* 1978, *J. Mol. Biol.* **119**, 237). The present study was designed to answer the question of whether X-ray diffraction could be used to usefully study the structure of fish corneas. This question has been answered affirmatively, and we report X-ray patterns from fish corneas. Furthermore, we note that our patterns and our structural interpretations do differ from the earlier study on beef corneas. In particular, we derive an estimate of the diameter of the collagen fibrils.

In the present study, the scleral cornea of the marine teleost toadfish and whole cornea of the elasmobranch skate were dissected out and put, with aqueous or vitreous humour, into the thin wall capillary tube. Slit collimated diffraction patterns were recorded for two sample orientations, perpendicular and parallel to the slit. The specimen-to-film distance was about 25 cm. The X-ray exposure time did not exceed 9 h.

We report that each cornea gave a fiber diagram. In the perpendicular case a strong but broad reflection, 670 Å for toadfish and 520 Å for skate, was observed as well as a sharp but weak reflection at 220 Å for both species. In the parallel case a very strong reflection, 660 Å for toadfish and 550 Å for skate, and a weak but broad reflection, 210 Å for toadfish and 200 Å for skate, were detected. In preliminary experiments on the partial air-drying of the fish corneas, the long X-ray spacing from the packing of collagen cylinders decreased noticeably.

The fiber diagram suggests that the strong reflection 670 Å and 520 Å for toadfish and skate arises from a packing of collagen cylinders while the 220 Å reflection is the third order of the 660 Å axial period of collagen. The first order reflection of the collagen axial period in the fish cornea was missing, as previously reported of beef cornea. It is tempting to interpret the weak extra reflections at 210 Å and 200 Å as arising from the Fourier transform of the collagen fibrils in the transverse direction. If we assume the collagen fibril is a solid cylinder with uniform density, a diameter of about 330 Å is then obtained for both collagen fibrils.

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Rapid pressure changes of the squid giant axon accompanied by prolonged action potentials. K. IWASA, AND I. TASAKI (National Institute of Mental Health, Bethesda, MD).

The mechanical changes in the squid giant axon during action potentials consist of two phases, swelling and shrinkage. Under normal conditions, the peak of the swelling coincides with the depolarization peak of the action potential, and the peak of the shrinkage with the hyperpolarization peak. In tetramethylammonium-ion-injected squid axons, which show prolonged action potentials, the initial phase is swelling, and its duration is comparable to that accompanied by the normal action potential. Immediately afterward, however, a prolonged shrinkage phase appears, while the axon is still in the depolarizing phase of the action potential. The shrinkage phase lasts as long as the prolonged depolarization phase. Because the swelling phase has a time course similar to the membrane impedance change, we attribute the first phase to the swelling of membrane proteins that determine the membrane impedance. We consider the shrinkage to be related to the calcium influx during excitation. Thus the pressure change observed is a synthesis of the two events taking place in different layers of the membrane. This interpretation is consistent with an observation that the external medium with low calcium-ion concentration reduces the shrinkage, whereas that with high calcium-ion concentration enhances it. Another important observation is that the pressure changes of tetraethylammonium-ion-injected axons are about $^1/_5$ of that of intact axons in both swelling and shrinkage. The reduction of the pressure changes is attributed to hydrophobic interactions of tetraethylammonium ions with membrane proteins.

The pressure sensor used was made with poly (vinylidene fluoride) film, a piezoelectric material, donated by Kureha Chemical Industry Co., Japan.

Orientation to uniform electric fields in the stringray Urolophus halleri: *sensitivity of response.* AD. J. KALMIJN AND VERA KALMIJN (MBL).

The electric sense of elasmobranch fishes aids the animals in prey detection and allows them, by electromagnetic induction, to gauge their drift in ocean currents and to establish their magnetic compass headings. To determine the elasmobranch's sensitivity to the electric fields induced by ocean currents, the marine stingray *Urolophus halleri* was trained to orient to strictly uniform voltage gradients.

Direct current was passed into the circular test tank by means of two stainless-steel electrodes. The stimulus current was divided uniformly over the animal habitat by the use of multiple salt-bridges. This method circumvents the adverse effects of electrode polarization, thus permitting precisely calibrated fields in the nanovolt/centimeter range. The stingrays were conditioned to procure food and to avoid punishment by swimming into an enclosure to the right relative to the field and not to enter a similar enclosure to the left with respect to the field. To eliminate the use of alternative cues, the electric field was reversed or kept the same from trial to trial in a random order, while the horizontal component of the earth's magnetic field was nulled with Helmholtz coils.

Three stingrays were tested for more than a year. Initial training took place in fields of 0.16 μV/cm to familiarize the animals with the procedure. The voltage gradients were successively lowered by factors of two. At each level, tests were run until the score indicated either a 3-out-of-4 or random orientation at a $P \leqslant .001$ significance level, as established by the statistical technique of sequential analysis. This technique was selected for its great power and economical use of the data.

All three stingrays were able to orient to fields of voltage gradients as low as 5 nV/cm (correct *vs.* total number of responses: 43/58, 45/61, and 72/104 respectively). This new threshold gradient is twice as low as was found through the heartbeat recordings of earlier years. The experiments also demonstrate the stingrays' ability to make meaningful use of the inanimate electric fields available in nature.

This work was conducted under contract with the Office of Naval Research, N00014-79-C-0071, as part of the authors' project on "Biological Sensors for the Detection of Electric and Magnetic Fields."

Identification of the active guanidinium group in saxitoxin. P. N. KAO, M. R. JAMES-KRACKE, C. Y. KAO (State University of New York Downstate Medical Center), C. F. WICHTMAN, AND H. K. SCHNOES.

Saxitoxin, one of several neurotoxins produced by certain *Gonyaulux* dinoflagellates, differs chemically from tetrodotoxin, but shares the specificity in blocking the sodium channel with a K_d of nanomols. Of the two guanidinium groups in saxitoxin, it is unclear which one or both, and whether the charged or uncharged form, might be responsible for biological activity. Using as end-points the residual sodium current (I_{Na}) in the space-clamped squid giant axon, as well as changes in the maximum rate of rise of the propagated action potential ($\dot{V}_{max}$), the potency of saxitoxin has been determined at different pH's. At 7.25, the dose of saxitoxin to reduce I_{Na} to 0.5 (ED_{50}) is 6 n*M*, and that to reduce $\dot{V}_{max}$ is 15 n*M*. At 8.25, the ED_{50} for lowering $\dot{V}_{max}$ is 26.5 n*M*, which is 1.77 times higher than that at 7.25. The 1,2,3-guanidinium deprotonates with a pK_a of 11.6; and the ratio of the concentrations of the charged form at 7.25 and at 8.25 is 1.00. The 7,8,9-guanidinium deprotonates with a pK_a of 8.25; and the corresponding ratio is 1.80 (0.9/0.5). Therefore, the observed potency ratio of 1.77 identifies the 7,8,9-guanidinium as the active group, and also that the charged form is involved in blocking the sodium channel.

Neosaxitoxin differs from saxitoxin only in having an $-OH$ group replace the $-H$ on N-1. The oxium group deprotonates with a pK_a of 6.75. Were the 1,2,3-guanidinium involved in biological actions, the presence of a negative charge so close to the normally positively charged guanidinium should have some influence. Yet, at pH's 6.50, 7.25, and 8.25, where the abundance of the negatively charged group range over *ca.* 60 fold, neosaxitoxin is essentially equipotent with saxitoxin. This observation further excludes the 1,2,3-guanidinium as contributing significantly to the blockage of the sodium channel.

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Pharmacological agents partially reproduce the effects of a circadian clock on the Limulus *lateral eye.* LEONARD KASS AND ROBERT B. BARLOW, JR.(Syracuse University, NY).

When the horseshoe crab is kept in constant darkness, the lateral eye produces larger electroretinographic responses (ERGs) at night than during the day. The elevated retinal sensitivity at night is mediated by efferent nerve fibers arising from a circadian clock in the brain (Barlow *et al.* 1977, *Science* **197**: 86–89). Part of the circadian change in ERG response is caused by changes in the structure of ommatidia; at night pigment cells move laterally, increasing the aperture; and photoreceptor cells (retinula cells) move distally, maximally exposing the rhodopsin-containing membrane to the incident light (Barlow *et al.* 1980, *Science* **210**: 1037–1039).

Octopamine (10 μM), forskolin (40 μM), and dibutyryl-cAMP (1 mM) injected subcorneally into the lateral eye *in situ* during the day increase ERG amplitudes and change the ommatidial structure to its nighttime state. Octopamine may be the efferent nerve transmitter; it is synthesized in the lateral eye and appears to be localized within the efferent nerve terminals (B. Battelle, National Eye Institute, NIH). Forskolin is believed to act directly on adenylate cyclase (Seaman *et al.* 1981, *PNAS* **78**: 3363–3367). Dibutyryl-cAMP presumably substitutes for cAMP's action on protein kinases. Our results provide further evidence for a possible role of octopamine as the efferent nerve transmitter.

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Anatomy of the ascidian tunicate tadpole, Ciona intestinalis. MICHAEL J. KATZ (Brown University).

Since 1866, when Kowalevsky first described its basic vertebrate body plan, the tunicate tadpole has frequently been suggested to be a descendant of organisms that were transitions between invertebrates and vertebrates. Such phylogenetic arguments have been largely based on 19th century anatomical descriptions of tunicate tadpoles. In general, these studies have not been repeated with modern histological techniques.

I examined newly hatched *Ciona* tadpoles (19 h after fertilization—18°C) in thick (stained with toluidine blue for LM) and thin (stained with uranyl acetate and lead citrate for EM) plastic sections. The 19 h tadpole is an active swimmer, and its simple anatomy is composed of six developed organ systems and four organ system rudiments. The developed organ systems are: (1) an acellular test completely covering the outside of the tadpole and forming the fins, (2) an epidermis, a single layer of generally cuboidal cells, (3) a dorsal tubular nervous system, (4) a notochord, (5) three muscle bands (each one-cell thick) extending along both lateral surfaces of the notochord, (6) three adhesive organs on the anterior end of the tadpole. The organ system rudiments are (1) a dorsal pharynx from which the incurrent siphon and the pharynx will develop, (2) bilateral atria, which will fuse and form the excurrent siphon, (3) a gut rudiment, (4) pockets of mesodermal cells which will form the adult musculature and circulatory system.

The tadpole nervous sytem can be subdivided into a rostral prosencephalon (recently described, *e.g.*, in Eakin and Kuda, 1971, *Z. Zellforsch.* **112**: 287–312) and a caudal deuterencephalon. Beginning in the caudal body at the head of the notochord, the deuterencephalon is a simple tube ringed by four nonneuronal cells with tight juctions along their lumenal edges: a capstone cell sitting dorsally, a cuboidal floor cell forming the ventral surface of the lumen, and two ciliated lateral cells. In the deuterencephalon, neurons are only found rostrally, and in these regions most axons are fasciculated medially below the floor cell. Caudally, most axons run along the ventrolateral edges of the two lateral cells. The vertebrate characteristics of the tadpole nervous system include: a dorsal neural plate, which rolls into a neural tube; axons running in marginal zones; ciliated ependyma-like cells lining the central canal; and axosomatic synapses.

Structurally, the tunicate tadpole is remarkably simple: there are few organs, and each organ is composed of few cells. The tunicate tadpole has many features of vertebrates, and thus it represents a special vertebrate organism in which to study the essence of organogenesis, uncomplicated by the variations and elaborations available in other organisms whose organs are constructed of many more cells.

Further studies on transmission by presynaptic spike-like voltage clamp depolarizations in squid giant synapse. R. LLINAS, M. SUGIMORI, AND S. M. SIMON (Dept. of Physiol. and Biophysics, NYU Medical Center, 550 First Avenue, NY, NY 10016.

Following pharmacological blockage of the g_{Na} (conductance) and g_K, the pre- and post-synaptic elements of squid giant synapse were simultaneously voltage clamped at their junction site (Llinás *et* *al.* 1979 *Biol. Bull* **157:** 380). The experimental design consisted of voltage clamping the preterminal element with voltage transients that replicated the original action potential recorded at the terminal before addition of TTX and 3 and 4 aminopyridine to the superfusion fluid and before TEA injection to the preterminal (Llinás *et al.* 1981, *Biophys. J.* **33:** 289–321). The present results allow a direct determination of I_{Ca} during the spike-like clamped voltage pulse and the direct measurement of postsynaptic current from resting level. The latency of I_{Ca} was a function of the duration of the action potential. The onset always occurred during the repolarizing phase of the spike. The amplitude of I_{Ca} was related to presynaptic voltage in a manner quite close to that predicted by a recent model for synaptic transmission at this junction (Llinás *et al.*, 1981 *Biophys. J.* **33:** 323–351). Modification of holding potential and of amplitude and duration of the voltage transient indicated that these variables play an important role in modulating synaptic transmission. The delay between onset of I_{Ca} and of the postsynaptic current was 375 ± 65 μsec and the relation between the amplitude of I_{Ca} and that of the post synaptic response varied between a first and second order value. The peak I_{Ca} was 300 $\mu A/cm^2$. The peak of the postsynaptic current varied from eight to fourteen μA. Calcium entry through g_{Na} does not generate a post-synaptic response, indicating that to release transmitter, calcium must enter via a specialized set of channels, probably associated with the active zone.

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On the origins of the slow PIII potential of the skate ERG. L. MEHAFFEY III (Vassar College), R. B. SZAMIER, R. L. CHAPPELL, H. RIPPS, L. M. HALVORSON AND D. J. TAATJES.

Several of the electrical potentials that make up the complex waveform of the vertebrate electroretinogram (ERG) are presumed to arise from non-neuronal retinal elements in response to changes in extracellular potassium $[K^+]_o$. For example, results obtained with K^+-selective electrodes have revealed a large, relatively slow, light-evoked decrease in $[K^+]_o$ in the region of the photoreceptor terminals, and intracellular recordings indicate that this ionic change acts upon the apical surface of the pigment epithelial cells to generate the so-called PI waveform (the cornea-positive component of the ERG c-wave). It has been suggested that the slow PIII potential (a cornea-negative wave that is almost a mirror image of the pigment-epithelial response) also result from the drop in $[K^+]_o$, but in this instance due to its action upon the retinal glia (Müller cells). In both instances, longitudinal current paths are created that are readily detected by trans-retinal electrodes.

We attempted in these experiments to examine the ionic and cellular bases of the slow PIII potential in skate (*Raja erinacea* or *R. oscellata*). Eyecup or isolated retina preparations were superfused with Ringer solutions containing drugs that (a) altered Müller-cell morphology, or (b) affected potassium conductance channels.

After isolated retina was bathed in Na-aspartate (50 m*M*) to block the activity of post-receptoral neurons, the slow PIII was the dominant potential in the ERG. Adding 25–50 m*M* DL-α-aminoadipic acid to the perfusate had no effect on the response, although this gliotoxin severely damaged Müller cells throughout the retina: EM study showed loss of glial cytoplasm and extensive disruption of the Müller-cell membranes. On the other hand, the slow PIII was suppressed fully by introducing barium (0.4 m*M*) to the aspartate-Ringer solutions. Moreover, when applied to the intact superfused eyecup, Ba^{++} blocked development of the PI (pigment epithelial) as well as the PIII components of the ERG. Since PI is a K^+-mediated response, this finding suggests that Ba^{++}, which interferes with K^+ conductance channels in other systems, exerts a similar effect on the skate retina.

In sum, the results indicate that intact Müller cells are not essential to the generation of slow PIII, but the response appears to be dependent upon light-evoked changes in $[K^+]_o$.

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The identification by lucifer injection of spectral inputs to the lamina cartridge of the dragonfly compound eye. I. A. MEINERTZHAGEN (Dalhousie University), R. MENZEL, AND G. KAHLE.

Four anatomical classes of photoreceptor axon have been described in the unit cartridge of the first optic neuropile, or lamina, of the visual system of *Sympetrum rubicundulum* (Armett-Kibel *et al.*, 1977, *Proc. R. Soc. B. Biol. Sci.* **196:** 385–413). To correlate these morphological classes with receptor spectral sensitivities, intracellular recordings were undertaken in this species in the same (ventral) eye region as that from which anatomical analyses were derived. Both KCl- and lucifer-filled electrodes were used.

Following lucifer injection, the eye was fixed and sectioned at 100 μm. Selected sections were re-embedded in Spurr's medium for re-sectioning at 2 μm to identify the injected cell. Receptor sensitivities of about 50 units fell into two classes: frequently sampled units with broad spectrum responses and less numerous units with narrow spectral-sensitivity functions. One unit recorded in the retina had a broad spectral sensitivity in the green and was identified as R1/4 in cross-sections of the basal ommatidium. Weaker filling of the neighboring cells R2 or R3 and R5 or R8 was also found, perhaps indicating dye-coupling. A similar broad-band spectral-sensitivity recording was made and injection filled R5/8, the other long retinular terminal class. A receptor recorded from a lamina site and having a narrow spectral sensitivity peak at 410 nm was obtained from a cell with an axon projecting to the medulla. This cell was assumed therefore to be long visual fiber R7. A second narrow spectral-sensitivity unit with a peak at 620 nm was recorded, and this cell was identified as R2/3. R1/4 was also filled. Its more weakly fluorescing profile extended through the lamina after that of R2/3 terminated in the distal third. Spectral sensitivities of lamina monopolar interneurons have also been recorded. In contrast to the broad-band sensitivity of LMCs reported by Laughlin (1976, *J. Comp. Physiol.* **112:** 199–211) a single marked small monopolar cell had an extremely sharp spectral-sensitivity curve peaking at 500 nm. Our results thus indicate the possibility of spectrally-encoded monopolar pathways.

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Axonal transport mechanisms and plasticity in hippocampal cortex of Rana catesbeiana. F. MORRELL (Rush Medical College), N. TSURU AND L. DETOLEDO-MORRELL.

Enduring enhancement of the efficacy of synaptic transmission in the hippocampal cortex of *Rana catesbeiana* has been demonstrated in a preparation developed at MBL (Morrell and Tsuru, 1974, *Biol. Bull.* **147:** 492). This enhancement was induced by brief (2 sec) tetanization of one hemisphere repeated every hour and was manifested by gradual increase in the duration of after-discharge (AD) and by the occurrence of spontaneous, massive, hypersynchronous, population spikes. Both these alterations began in the stimulated hemisphere and gradually spread to the contralateral (2°) cortex. Once established at the 2° site, the altered electrical activity became independent of events in the stimulated hemisphere and persisted indefinitely despite cessation of stimulation and even complete ablation of the formerly stimulated hemisphere. Thus, the cells of the 2° hemisphere had acquired a long-lasting change in excitability and in other synaptic properties as a consequence of prolonged exposure, via normal synaptic linkages, to cells driven by repeated electrical stimuli. The strict anatomical constraints, necessity for intermittent stimulation, and the time requirement suggested two alternative mediating mechanisms: (1) frequent bombardment by action potentials transmitted from stimulated to 2° region and (2) axonal transport of some molecular species affecting pre- and post-synaptic elements of the contralateral (2°) cortex.

To distinguish between these possibilities, we adopted a technique of Robert and Oester (1970, *J. Pharmacol. Exp. Ther.* **174:** 133–140) that allowed continuous bathing of the hippocampal commissure with agents effective against one but not the other of these putative mechanisms. A silastic strip containing 20% lidocain base or 0.1 mg colchicine was implanted directly on the commissure. The drugs leech slowly out of the silicone, perfusing immediately adjacent structures. Lidocaine at these concentrations blocks action potentials without affecting transport, whereas colchicine prevents axonal transport without interfering with conduction of nerve impulses (Albuquerque *et al.* 1972, *Exp. Neurol.* **37:** 607–634). Surgical transection of the commissure (eliminating both processes) and a blank silastic (affecting neither process) served as controls.

Only colchicine-treated animals failed to develop modification of excitability and synaptic responsiveness in the 2° hemisphere. Axonal transport, therefore, appears essential to the plasticity of hippocampal synapses.

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Circadian changes in visual sensitivity of Limulus: *Behavioral evidence.* MAUREEN K. POWERS (Vanderbilt University, Nashville, TN) AND ROBERT B. BARLOW, JR.

Physiological and anatomical measures show that the sensitivity of *Limulus* lateral eyes fluctuates daily: at night, single photoreceptors are more sensitive, amplitude of the electroretinogram increases, and the pupillary aperture enlarges. These changes are controlled by a circadian clock which is located in the brain and transmits efferent nerve activity to the lateral eyes. We report here that the animal's response to light also fluctuates daily and that the magnitude of change in behavioral threshold corresponds to the magnitude of change in physiological threshold.

Freshly collected male *Limuli* were securely restrained in an aerated seawater aquarium located in a lightproof shielded cage. Pilot work had shown that current pulses delivered to the fleshy hinge area between anterior and posterior carapace produced an unconditioned change in the rate or amplitude of tail and gill movements. Tail movements were monitored by attaching a bead thermistor to the middle of the tail, and gill movements were monitored by placing a thermistor underneath the animal. After 12–24 h habituation to the apparatus, a classical conditioning procedure was used to associate a 10 sec light stimulus to the lateral eye with shock once every 5–10 min until the animal responded to the light. Nine out of fourteen *Limuli* responded satisfactorily within 50 trials. Animals were generally less responsive if collected more than 7 days before the experiment.

Visual sensitivity was measured in 5 trained *Limuli* during the day and the night, following at least 24 h in darkness, by presenting light of different intensities and computing the percent response to each intensity. In every case, behavioral responses were elicited during the night with stimuli that had been below threshold during the day. The three animals with the most complete data were 1.0–1.5 log units more sensitive at night than during the day, which is about the difference in sensitivity observed in physiological experiments.

Our results are the first to demonstrate a behavioral effect on the circadian rhythms within the *Limulus* visual system. The data show that there is a strong correspondence between behavioral and physiological changes in sensitivity, and they imply that the efferent input from the brain to the lateral eyes plays a significant role in regulating the visual sensitivity of the behaving animal.

Supported by a URC grant from Vanderbilt University, and by NIH grants EY03352, EY0667 and NSF grant BNS 7719436.

Form birefringence contribution to the voltage-dependent optical retardation of squid axon membrane. E. QUINTA-FERREIRA AND D. LANDOWNE (University of Miami).

Squid axons were internally perfused and voltage-clamped using standard techniques. The axons were mounted at 45 degrees to crossed polars to measure their optical retardation. A change in membrane potential of 80 mV is associated with a change in optical retardation of about one part in ten thousand. Signal averaging was used to enhance the signal to noise ratio.

To distinguish between intrinsic and form birefringence contributions to the change in optical retardation associated with voltage pulses, axons were perfused with solutions containing added sucrose to raise their index of refraction (n). With 1.8 *M* sucrose in the internal perfusion fluid ($n = 1.43$) the retardation response to a voltage step was about one half the size of the response with 0.3 *M* sucrose inside ($n = 1.36$). With 2.5 *M* sucrose ($n = 1.46$) the response was very small and appeared inverted. These changes were reversible: the responses become larger again on returning to low sucrose. High sucrose reduced the response for depolarizing and hyperpolarizing responses proportionately. The shape of the responses did not change dramatically, although there was a suggestion of a slight slowing in high sucrose.

These results indicate that a large fraction of the observed retardation response is due to form birefringence. With modified Weiner equations and this data, the predicted minimum of the form birefringence curve is at a membrane index of refraction of about 1.56. This suggests protein involvement in the signal, as n is about 1.48 for most lipids. A minimum at $n = 1.56$ would be of the opposite sign of the signal in low sucrose ($n = 1.36$), implying a contribution of intrinsic birefringence.

Supported by NIH research grant NS 13789. E. Q. -F. Supported by Calouste Gulbenkian Foundation.

Aplysia neurons R3–R14: Electrophysiological tracing of their peripheral axons. ANN R. RITTENHOUSE (Boston University) AND CHRISTOPHER H. PRICE.

The cell bodies and axons of identified neurons R3–R14, located in the parietovisceral ganglion, are easily identified by their large size and distinctive white color. Earlier anatomical work showed that these cells project axons into the branchial nerve and presumably terminate in the region surrounding the heart. We have confirmed this by visually following pathways under a dissecting microscope and by electrophysiologically tracing them, by matching, one for one, spontaneous and evoked intracellular soma spikes with extracellular axonal potentials recorded by suction electrodes.

White fibers from R3–R14 travel through the branchial ganglion, along the length of the right and left efferent vein nerve and in the auricular branch of the pericardial nerve. R14 alone sends a process into the pericardial nerve and branches into the ventricular and auricular nerves. But it does not enter the heart. From all of these nerves, white cell axons arborize in the reno-branchial veins and their cross muscles, throughout the muscle layer surrounding the heart, in the heart, and on the surface of the kidney. Large white varicosities were seen along axons in all of these tissues. Individual cells were mapped electrophysiologically to all of the above tissues.

Sawada and his colleagues have shown that R14 also sends axons in the vulvar nerve to the anterior aorta and that electrical activity of R14 modulates aortic contractions. Our work shows that the twelve R3–R14 neurons send axons to vascular tissues of many types in a variety of locations. This suggests that like R14, cells R3–R13 may be modulatory neurons for vascular smooth muscle, or may be neurosecretory (as proposed in earlier work), or both.

Work supported in part by DHHS(NIH) grant NS 16399.

Optical measurement of potential changes in axons and processes of neurons of a barnacle ganglion. WILLIAM N. ROSS AND VICTOR KRAUTHAMER (New York Medical College).

Optical recordings of transmembrane voltages at multiple sites on single cells, combined with standard intracellular recording and staining methods, makes possible an analysis of the regional properties of neurons. We have used this combination of techniques to examine cells of the supraesophageal ganglion of the giant barnacle, *Balanus nubilus.*

Ganglia were mounted on the stage of a Zeiss Universal Microscope, stained with the voltage-sensitive dye NK2367, and imaged with a ×40 water-immersion lens onto a 6 × 6 square array of photodiodes. Each element corresponded to 40 × 40 μm^2 in the object plane. Several large cells along the anterior-medial margin of the ganglion were selected for examination. Lucifer Yellow injections of these cells showed that each of these cells sent 10–15 μm axons out the ipsilateral antennular nerve and arborized in the ipsilateral neuropil. The cells were impaled with a microelectrode and stimulated alternately with square depolarizing and hyperpolarizing current pulses. The depolarizing pulse evoked an action potential, which was recorded with a suction electrode on the antennular nerve. The resulting changes in absorption, detected by each photodiode element at 720 ± 25 nm, were averaged on a laboratory computer.

Optically detected action potentials could be seen on each photodiode positioned over the cell body and along the axon. Signals corresponding to the electrotonic spread of hyperpolarizing pulses were also detected on these elements. Appropriately, they were smaller in amplitude and had a slower time to peak with increasing distance from the soma. In addition, clean signals from neuropilar processes 5 μm or less in diameter were recorded. The shape of the action potential and hyperpolarizing potential were different from those recorded from the axon at equivalent distances from the soma, suggesting that these processes had different electrical properties than the axons.

Supported in part by UPHS grant NS16295 and the Irma T. Hirschl Foundation.

Ultraviolet responses of the Limulus *median ocellus.* LARRY A. WESTERMAN (Syracuse University) AND ROBERT B. BARLOW, JR.

The median optic nerve of the horseshoe crab, *Limulus polyphemus,* discharges nerve impulses in response to ultraviolet illumination of the median ocellus, *in situ.* The impulses are conducted by only about 10% of the 300 nerve fibers from the twin ocelli. The remaining nerve fibers are silent in the cut nerve preparation. The spectral sensitivity of single ocellar nerve fibers was determined from threshold responses to 5-sec flashes of narrow-band chromatic stimuli. The spectral sensitivity function reaches a maximum at 380 nm. The incident energy density at the corneal surface for a threshold response of 1 spike/sec is 2×10^{-7} $\mu W/cm^2/nm$ at 380 nm (stimulus bandwidth 10 nm). The ocellar response is more than 5 log units less sensitive at 520 nm. The electroretinographic response of the ocellus also reaches maximum sensitivity in the UV (λ_{max} 360–380 nm), but in addition shows a secondary maximum, 1.5–2.5 log units less sensitive, at 520 nm. No similar visible sensitivity is recorded from single ocellar nerve fibers.

Intensity coding in the optic nerve discharge is graded over a range of at least 8 log units of light intensity. The steady-state firing rate in response to long (10-sec) stimulu delivered to the dark-adapted ocellus is approximately linear when intensities are near threshold. The rate reaches a sloping plateau at 3–4 log units above threshold, and then positively accelerates for intensities greater than 5–6 log units above threshold. In one ocellar nerve, the firing rate did not saturate for incident flux up to 3.7×10^{14} photons/(sec·cm^{-2}) at the corneal surface (380 nm) which produced a steady-state discharge of 51 spikes/sec.

At night the sensitivity of the lateral eye of *Limulus* is increased by the action of a centrally-located circadian clock. Previous work shows that illumination of the ocellus increases the efferent optic-nerve activity generated by the clock. The spectral sensitivity of the enhancement effect matches that of ocellar nerve response over the wavelength range of 300–640 nm. We conclude that the median ocellus functions primarily as a UV receptor that can enhance the sensitivity of the lateral eye at night.

Research supported by NIH grant EY00667 and NSF grant BNS-7719436.

An interpretation of the low-angle X-ray data from squid retina. C. R. WORTHINGTON (Carnegie-Mellon University) and A. R. CZETO.

We have studied the molecular structure of the invertebrate rhabdomeric visual cells of squid by low-angle X-ray diffraction. It is known from previous X-ray studies (1980, *Biol. Bull.* **159**, 482) that X-ray patterns can be obtained from 1 mm strips of intact untreated squid retina mounted in a specimen chamber with excess Ringer's solution. X-ray patterns showing diffraction orders 2–8 of $d \approx 620$ Å were reported. We have now recorded the first order reflection using slit collimation and a long specimen-to-film distance of 25 cm. The present patterns are consistent with a two-dimensional hexagonal array of microvilli which has a rotation axis about the (11) direction. The rotation axis is coincident with the light direction. We find that the microvilli arrays in our preparations have considerable disorder. Moreover there is wide variation between retinas. Thus, not all X-ray patterns show well-defined reflections. On the other hand, the intensity transform profile of the microvilli arrays appear to be the same for all intact untreated squid retina patterns. The observed intensity transform has a minima (but not a zero) centered at a Bragg spacing of 125 Å while there are two zeros at 62 Å and at 37 Å respectively. If the microvilli arrays consisted of cylindrical tubes arranged at the corners of a hexagonal lattice, and if such cylindrical tubes had a wall thickness of a single membrane, then, from diffraction theory, the intensity transform of this model would resemble the transform of a single membrane. However, this single membrane is not observed. The observed transform profile can be interpreted as arising from a pair of membranes. Thus, the membranes of adjacent microvilli adhere together to form a membrane pair. The separation between adjacent membranes is about the same as in vertebrate discs. In our preparations, the microvilli membranes are markedly asymmetric, with extra material on the outside of the microvilli; and they had a width of about 80 Å.

This grant was supported by NIH grant NS 09329.

PARASITOLOGY

Identification of Leishmania *species by restriction enzyme digests of kinetoplast DNA* D. MCMAHON PRATT AND D. WIRTH (Harvard University, Biological Laboratories).

Kinetoplast DNA was isolated from the promastigotes of several species of *Leishmania* by isopynic centrifugation in CsCl. The DNA was digested with the restriction enzymes Hae III and Hpa II and analyzed by electrophoresis in agarose. Distinct restriction enzyme fragments were obtained from the various species of *Leishmania* (*L. tropica, L. donovani, L. enriettii*). Southern blot analysis using ^{32}P-labeled *L. mexicana* kinetoplast DNA showed hybridization only with homologous *L. mexicana* DNA isolated either from promastigote kinetoplast DNA or from total amastigote DNA. This specific hybridization may be useful in identifying *Leishmania* species.

Comparative studies on knob positive and knob negative clones of Plasmodium falciparum *malaria.* KAMINI MENDIS, CHRISTIAN OCKENHOUSE, MAGGIE SO, MATS WAHLGREN, ELLEN WINCHELL, ROBERT ALLEN, RICHARD CARTER, MARCEL HOMMEL, LOUIS MILLER, WILLY PIESSENS, JOHN SCHMIDT, IROKA UDEINYA, *PETER DAVID (*Department of Medicine, Harvard Medical School, and Division of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts, 02115).

Membranes of erythrocytes infected with *Plasmodium falciparum* develop protrusions called knobs which are thought to have a function in the sequestration of maturing parasites in tissue capillaries.

We have compared two clones of parasites isolated from the West African strain of *P. falciparum* FCR3; the K^+ clone produces knobs and the K^- clone does not.

Video Enhanced Contrast, Differential Interference Contrast (AVEC-DIC) microscopy showed a granular aspect of the infected erythrocyte membrane with K^+ and not with K^- parasites.

Metabolic labeling of the K^+ and K^- parasites followed by polyacrylamide gel electrophoresis showed differences between the two clones. The differences were also detected by immunoprecipitation using human immune serum.

The study of surface antigens by the Indirect Immunofluorescent Antibody Test, using human immune sera from the Gambia and from Indonesia, and as antigen, unfixed erythrocytes parasitised by K^+, K^-, Ugandan and Thai strains, showed that knobs were not the only immunogenic structures on the surface of the infected erythrocytes and that inter-strain antigenic variation occurred. Furthermore, immunoprecipitation showed that different antigens were precipitated by the Gambian and Indonesian sera.

In relation to the interaction between infected erythrocytes and host cells during parasite sequestration, we studied the binding *in vitro* of infected erythrocytes to human monocytes and to human melanoma cells (C37 CRL 1587 cell line). Binding occurred with K^+ and not with K^- parasites, and was inhibited by immune serum or by treatment of the infected cells with Trypsin or Chymotrypsin but not with Neuraminidase. Bound cells were examined by scanning electron microscopy. Results of *in vitro* binding assays were correlated with parasite sequestration *in vivo* in the primate host *Saimiri sciureus.*

Lectins discriminate between pathogenic and non-pathogenic South American trypanosomes ISABEL K. F. DE MIRANDA SANTOS AND MIERCIO EXPEDITO A. PEREIRA (Tufts University, Department of Medicine, Division of Geographical Medicine).

Cell surface carbohydrates of *Trypanosoma cruzi, T. rangeli,* and *T. conorhini* were analyzed by a micro-agglutination assay employing 27 highly purified lectins and by binding assays using various ^{125}-labeled lectins. The following five lectins discriminated between the trypanosomes: (1) tomato lectin (an N-acetyl-D-glucosamine-binding protein), both in purified form and as crude tomato juice, (2) *Bauhinea purpurea* lectin (an N-acetyl-D-galactosamine-binding protein), which selectively agglutinated *T. cruzi*; (3) *Vicia villosa* lectin (an N-acetyl-D-galactosamine-binding protein which was specific for *T. rangeli*; (4) peanut (a D-galactose-binding protein), and (5) *Ulex europaeus* (an L-fucose-binding protein) lectins which reacted only with *T. conorhini.* Binding studies with ^{125}I-labeled lectins were performed to find whether unagglutinated cells of the three different species of trypanosomes might have receptors for these lectins, in which case absence of agglutination could be due to a peculiar arrangement of the receptors. These assays essentially confirmed the agglutination experiments.

These studies offer insight into the molecular mechanisms at play at the host-parasite interface, as *T. cruzi* is pathogenic to mammals; and *T. rangeli* and *T. conorhini*, while capable of infecting mammals, do not cause disease but apparently only a transient parasitemia. Diagnostic problems that arise between the latter protozoa and *T. cruzi* due to similar morphology and insect vectors can be easily circumvented by simple agglutination techniques such as those employing purified lectins or, in the case of field studies, even crude tomato juice.

Work done in the Biology of Parasitism Course.

Immune evasion by Schistosoma mansoni. R. A. SIDNER, M. WAHLGREN, E. AVILA, L. A. UNBEKANT, C. OCKENHOUSE, M. SO, K. MENDIS, E. WINCHELL, L. GREENBAUM, D. HARN, G. MOSER (Harvard Medical School).

Lung stages of *Schistosoma mansoni* evade host immune defenses by at least three mechanisms: aquisition of host antigens, loss of surface antigens, and intrinsic membrane changes which preclude immune-system damage. In a preliminary report given here last year, Klotz *et al.* (*Biol. Bull.* **159**: 496) stated that immune evasion by 5-day-old lung schistosomula did not occur in C57/Bl6J mice. To clarify this matter we immunized C57/Bl6J and CBA/J inbred mice by percutaneous exposure to 500 irradiation-attenuated cercariae each. Seven weeks later, these mice and age-matched, unimmunized controls were each challenged either percutaneously with 200 normal cercariae or intravenously with 200 skin-derived or 200 5-day-old lung-derived schistosomula. Seven weeks post-challenge, mice were killed with Na pentobarbital and adult worms recovered by perfusion.

For C57/Bl6J mice, we recovered 15.8 ± 10 worms from 10 cercariae-challenged animals versus 51.9 ± 40.7 from 10 control mice. Nine mice challenged with skin-derived schistosomula gave 4.6 ± 6.1 worms, compared to 24.5 ± 24 from eight control mice. Eight mice challenged with lung-derived schistosomula yielded 63 ± 44 worms, while nine controls gave a similar number (85.1 ± 65).

For CBA/J mice, we recovered 35 ± 17 worms from 7 cercariae-challenged mice and 61 worms from the one surviving unimmunized control. Six mice challenged with skin stages gave 3.2 ± 2.2 worms, compared to 17.7 ± 21.2 from controls. Ten mice challenged with lung stages yielded 43.2 ± 45.9 worms, while recovery from nine control animals was insignificantly different (62 ± 58.6). Clearly, immunization afforded both mouse strains protection against cercariae and skin-derived schistosomula, but 5-day-old lung-derived schistosomula effectively evaded host immune defenses.

Notes on the life-cycle of Lacistorhynchus tenue *(van Beneden), 1858) (Cestoda: Tetrarhynchidea).* HORACE W. STUNKARD (The American Museum of Natural History, New York, N. Y. 10024).

Linton (1889, *Ann. Rep. U. S. Comm. Fish.*, p. 486) reported *Rhynchobothrium tenuicolle* from the spiral valve of *Mustelus canis* at Woods Hole, Massachusetts. He (1890, *idem.*, 825–829) described the parasite as a new species, *Rhynchobothrium bulbifer.* The specific name *R. bulbifer* was suppressed as a synonym of *Lacistorhynchus tenuis* (van Beneden, 1858). Yamaguti predicated that the Greek word, *rhynchos*, is a neuter noun and accordingly, he changed the specific name from *tenuis* to *tenue.* The proglottids detach from the strobila before they are mature, develop anterior adhesive structures, and complete their development. When gravid, the proglottids are mere sacs, filled with eggs. When the proglottids are placed in sea water, the eggs are extruded in a stream through the uterine pore. Thousands are shed in a few minutes. The eggs are oval, average 0.054 by 0.036 mm in diameter, are operculate, and are not embryonated when voided. In 4 days at room temperature, the oncosphere-larvae are active in the eggs. A day or two later the opercula open and the coracidia are swimming. They are ingested by copepods, *Acartia tonsa*; copepods placed in a bowl of swimming larvae eat them in great numbers. The oncospheres bore out of the intestine of the copepod to reach the body cavity. Stunkard (1977, *Biol. Bull.* **153:** 387–412) described plerocercoid metacestodes of *L. tenue*, encysted in the stomach-wall of squids, *Loligo pealeii*, at Woods Hole. A second intermediate host in the life-cycle, presumably a small fish, eats the copepod and in turn is eaten by larger fishes and squids. These animals are paratenic hosts that carry the juveniles and transmit the parasite to *M. canis.* Linton (1924, *Proc. U. S. Nat. Mus.* **64:** 1–114) reported encysted plerocercoids of *L. tenue* in 35 different species of marine teleosts.

Investigation supported by NSF-DEB-80-06150.

Molecular analysis of gene regulation in protozoan parasites. D. WIRTH (Harvard University), R. CARTER, G. CROSS, P. DAVID, C. FRENCH, M. HOMMEL, L. MILLER, D. MCMAHON PRATT:

Genomic library of Leishmania enriettii *DNA.* DNA was isolated from *L. enriettii* promastigotes digested with the restriction endonuclease Pst I and cloned into the plasmid pBR322. We observed a transformation frequency 10^3 transformants/μg of DNA. More than 80% of these transformants contained segments of *L. enriettii* DNA. This genomic library was screened with ^{32}P-labeled *Drosophila* tubulin by colony hybridization. Six positive clones were found. These are currently being characterized.

Isolation and in vitro *translation of malaria mRNA.* Parasite mRNA isolated from the erythrocytes of *P. chabaudi* infected mice was translated in the reticulocyte cell-free system. These *in vitro* translation products were compared to infected cell proteins labeled *in vivo* with ^{35}S-methionine from the same erythrocytic stages. SDS polyacrylamide electrophoresis and autoradiography showed similar patterns of labeled proteins from the *in vitro* and *in vivo* labeling, including some apparently stage specific proteins.

Variant surface gene expression in Trypanosome brucei. African trypanosome infection is characterized by waves of prasitemia in the infected animal. Trypanosomes isolated at the peak of each wave express a distinct cell-surface antigen. Each antigen is encoded by a different gene. In a trypanosome expressing a certain antigen, the gene for that antigen is duplicated and moved to a new genomic location for expression. By Southern blot analysis, we were able to demonstrate both gene duplication and movement to an expression site for *T. brucei* expressing variant surface antigen 221.

Continued from Cover Two

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Continued on Cover Three

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CHOLINE REQUIREMENT OF THE MICROCRUSTACEAN *MOINA MACROCOPA*: A PURIFIED DIET FOR CONTINUOUS CULTURE

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ABSTRACT

Under axenic culture conditions, choline is a required nutrient of the microcrustacean *Moina macrocopa*. Lecithin (phosphatidylcholine) as a component of an artificial biphasic diet serves as an efficient source of choline. *Moina* can synthesize choline efficiently via methylation of dietary ethanolamine. The animals assimilate dietary choline 10 times more efficiently from a particulate source than from a soluble one. Liver infusion, the only undefined component of the artificial medium, contains 1% choline and contributes substantially to choline's availability. The liver infusion can be adequately replaced by an increase in dietary particulate choline or soluble choline. As a result, the artificial medium for the growth and continuous reproduction of *Moina* is now completely defined. *Moina macrocopa*'s requirement for choline in a particulate form is estimated to be 750–850 mg/100 g diet at a culture temperature of 26°C.

INTRODUCTION

Lecithin (phosphatidylcholine) is an important nutrient for crustacean growth and metabolism. Lester *et al.* (1975) showed that solubilization of cholesterol was effected by N-(N-dodecanosarcosyl) taurine (DST), a model of the type of detergents synthesized by crustaceans, and that the process is more efficient in the presence of lecithin. Kanazawa *et al.* (1979) showed that certain lecithins incorporated into an artificial diet for the prawn *Penaeus japonicus* enhanced growth.

In the lobster *Homarus americanus*, Conklin *et al.* (1980) eliminated mortality associated with juvenile molting by including soy lecithin in a purified diet. D'Abramo *et al.* (1981) showed that the active ingredient of the soy lecithin was phosphatidylcholine (PC), and suggested that the lecithin molecule was associated with a lipoprotein that efficiently transported cholesterol from the hepatopancreas to the hemolymph. In a continuation of these studies, we designed the present work to analyze the effect of lecithin on the growth of the microcrustacean *Moina macrocopa* cultured axenically on an artificial diet. As choline is a general requirement of insects (Dadd, 1970), we concurrently investigated the dietary contribution of choline from lecithin and the possible choline contribution of liver infusion, the only undefined ingredient of the artifical diet used.

MATERIALS AND METHODS

Moina macrocopa, a freshwater crustacean of the order Cladocera, was grown axenically on an artificial diet. The culture medium was biphasic, composed of a particulate and a soluble phase. It had only one undefined component: liver infusion (Oxoid). This diet (the control) has supported continuous growth and reproduction

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Abbreviations: PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

of several strains in our laboratory for over 4.5 years (approximately 162 generations). The diet's constituents are listed in Table I. The sources of ingredients and procedures of formulation are described elsewhere (D'Abramo, 1979). Total particulate concentration was 20.30 mg%. The egg lecithin component of the diet was associated with lipid-protein particles (particulate phase) and was provided at a concentration of 0.75 mg%. Known choline sources were the lecithin and the vitamin mix, which provided 0.14 mg% choline in the form of choline dihydrogen citrate.

Various additions, deletions, and substitutions to the control diet were made to investigate the nutrient contribution of lecithin. The nutritive quality of these various diets was evaluated by comparing biomass of populations grown from a single parthenogenetic female (strain L1) inoculant. First instar females were aseptically transferred from the control medium (Table I) to the various test media. All populations were grown at 26°C in near total darkness in 20 × 125 mm screw cap culture tubes containing 10 ml of medium. Animals were exposed to light only during the daily suspension of particles via a vortex mixer, and during determinations of the time when the original inoculant released her first brood. The initial particle concentration in the culture tubes was the same (380×10^3 ml^{-1}) for all media, since particle ingestion rates depend on ambient concentration (D'Abramo, 1980).

Initially each of three culture tubes containing an experimental diet was inoculated with a single female. To eliminate some variability, day 1 of population growth was designated as the day on which an original female inoculant released her first brood of young. When populations had developed, three animals from each tube were used as subsequent inoculants. Sequential transfers continued until 20 populations (5 harvested on each of days 7, 8, 9, and 10) were collected. The

TABLE I

Composition of control diet as per cent w or v/v. Total particulate = 20.30 mg%.

Soluble phase		Particulate phase	
KCl	3 mg	Cholesterol	0.6 mg
$MgSO_4 \cdot 7H_2O$	4 mg	SA gel[4]	3.0 ml
Ca (as Cl^-)	2 mg	FV particles[5]	1.0 ml
Glycylglycine	50 mg		
K_3PO_4	2 mg		
$Na_2SiO_3 \cdot 9H_2O$	2 mg		
Fe (as NH_4^+citrate)	0.05 mg		
Metal mix L[1]	1 ml		
Nucleic Acid mix V[2]	2 ml		
Oyster glycogen	10 mg		
Vitamin mix M1B[3]	2 ml		
Liver infusion	40 mg		

[1] 1 ml = $Na_2EDTA \cdot 2H_2O$, 3.81 g; Zn (as $SO_4^{=}$), 0.30 mg; B, 0.12 mg; Mn (as Cl^-), 0.087 mg; Fe (as $NH_4SO_4^{=}$), 0.06 mg; Co (as Cl^-), 0.024 mg; Cu (as $SO_4^{=}$), 0.024 mg; Mo (as NH_4^+), 0.036 mg.

[2] 1 ml = adenylic acid, 20 mg; guanylic acid, 10 mg; cytidylic acid, 10 mg; thymidine, 10 mg.

[3] 1 ml = thiamine HCl, 0.5 mg; nicotinamide, 1.5 mg; pyridoxine HCl, 0.2 mg; biotin, 0.06 mg; putrescine·2HCl, 0.1 mg; vitamin B_{12}, 0.002 mg; choline H_2citrate, 0.2 mg; riboflavin, 0.2 mg; folic acid, 0.1 mg; Ca pantothenate, 4 mg.

[4] 1 ml = rice starch, 10 mg; 2× crystalline egg albumin, 4.10 mg.

[5] 1 ml = 2× crystalline egg albumin, 8 mg; egg lecithin, 0.75 mg; BHT (butylated hydroxytoluene), 1 mg; ergocalciferol, 0.66 mg; retinolpalmitate, 0.25 mg; palmitic acid, (16:0) 1 mg; oleic acid (18:1), 0.3 mg; linoleic acid (18:2), 0.7 mg; linolenic acid (18:3), 1 mg.

sequential transfer procedure diminished carryover of nutrients from the original control medium to the experimental medium.

When populations were harvested, animals were immediately counted, sized, and categorized. The categories were: female instars I–IV, adult parthenogenetic females, adult gamogenetic females, and adult males. Biomass of each population was determined from a length–dry weight relationship derived previously (D'Abramo, 1979). The number of animals that had died, as indicated by body distortion, was also recorded. Diets were compared by the average biomass of all 20 populations (composite biomass) harvested during the four day period. Statistical analysis employed two-way ANOVA and multiple comparison Scheffe tests (Zar, 1974). Standard error of mean values were calculated from the error mean square value derived from the ANOVA. Sets of dietary treatments were designed after examination of previous results. As the number of treatments increased, various groups were arranged as separate experiments for ease of comparison. Experiment I evaluated the effect of the egg lecithin (70% PC, ICN Nutritional Biochemicals, Cleveland, OH) component of the particulate portion of the diet. Quantities of lecithin were 0.0, 0.25, 1.00, and 1.50 mg% (control = 0.75 mg%). In experiment II, pure phospholipids (egg PC, soy PC, soy phosphatidylinositol (PI), and egg phosphatidylethanolamine (PE) (Sigma Chemical Co., St. Louis, Mo.)) were substituted for the egg lecithin of experiment I. These phospholipids were added at 0.5 mg%, since the control diet had egg lecithin that was 70% pure. In experiment III (diets B–F, Table II), lecithin was eliminated from the diets and the combined effects of increased fat in the particles and increased choline from the vitamin mix were analyzed. To compensate adequately for the calorific contribution of the lecithin, the fatty acid component of the particles of the diet was increased from 3.0 to 4.5 mg%. The choline in the vitamin mix (soluble component) was increased from 0.14 to 0.49 to 0.70 mg%. Experiment IV (diet A, Table III) evaluated the liver infusion's contribution with both particulate and soluble sources of choline absent. Total fat content of the particles remained at 4.5 mg%.

The choline in the liver infusion, the only undefined component of the maintenance medium, was analyzed quantitatively according to the method of Lim and Schall (1964). Experiment V (diets B–G, Table III) substituted for the liver infusion ingredient by increasing particulate and soluble choline sources, singly or in combination. In one sequence (diets B, C, G) the concentration of soluble choline as choline dihydrogen citrate was increased from 0.14 to 0.7 to 1.75 mg%, while the concentration of the pure soy lecithin in the particulate phase remained at 0.5 mg% (0.069 mg% choline). Soluble choline levels of 1.40 and 2.00 mg% (diets D and

TABLE II

Effect of increases in soluble choline on the composite biomass and mortality of 20 harvested populations of Moina macrocopa *grown on artificial diets. Liver infusion constant at 40 mg%.*

Diet	Fatty acid concentration	Soluble choline (mg%)	Particulate choline (mg%)	Biomass (μg) ± SD	% Mortality
A	3.0	0.0	0.075	716 ± 253	4.5
B	3.0	0.0	0.0	463 ± 238	16.1
C	4.5	0.0	0.0	619 ± 267	18.7
D	4.5	0.14	0.0	442 ± 178	18.2
E	4.5	0.49	0.0	883 ± 293	7.8
F	4.5	0.70	0.0	916 ± 102	4.2

TABLE III

Effect of no dietary choline, and substitution of the choline of the liver infusion with soluble and particulate sources of choline (singularly and in combination), upon composite biomass and mortality of 20 harvested populations of Moina macrocopa *grown on artificial diets. Liver infusion absent. Particulate source of choline was pure soy lecithin.*

Diet	Fatty acid concentration (mg%)	Soluble choline (mg%)	Particulate choline (mg%)	Biomass (μg) ± SD	% Mortality
A	4.5	0.0	0.0	387 ± 212	36.6
B	3.0	0.14	0.069	598 ± 348	30.0
C	3.0	0.70	0.069	979 ± 190	1.2
D	4.5	1.40	0.0	946 ± 188	3.0
E	2.5	0.0	0.138	742 ± 236	4.4
F	4.5	2.00	0.0	758 ± 168	2.8
G	3.0	1.75	0.069	614 ± 139	4.1

F) were substituted for the particulate sources of choline. Finally, all sources of soluble choline were removed and pure soy PC was increased to 1.0 mg% (0.138 mg% choline, diet E). The amount of free fatty acids normally added to the particles was decreased proportionate to the quantity and quality of the fatty acids introduced through the increase in soy PC.

RESULTS

In all experiments the effect of harvest day on *Moina* population biomass was significant ($P \leq 0.01$). In some cases (experiments I and II) this factor contributed to large standard deviations within the average biomass of the twenty harvested populations grown on each diet. For experiments I and II standard error of mean values for each diet were 72.8 μg and 112.9 μg respectively.

Experiment I

Eliminating the egg lecithin component from the control diet significantly reduced productivity ($P \leq 0.01$). Growth rates on the diet containing 1.5 mg% egg lecithin were significantly ($P \leq 0.01$) higher than those of the other diets. On any harvest day, biomass generally increased as the amount of egg lecithin in the diet increased from 0.0 to 1.5 mg% (Fig. 1).

Experiment II

Growth increased when the 70% pure egg lecithin of the control diet was replaced by pure phospholipid sources (egg PC, soy PC, and egg PE). The egg PC and soy PC were significantly ($P \leq 0.01$) better than the control diet. The pure soy PI substitute, however, produced poor growth and high mortality. Growth with the dietary egg PC was significantly greater ($P \leq 0.01$) than that associated with the egg PE (Fig. 2).

Experiment III

Adding free fatty acids to compensate for the calorific loss of the lecithin deletion (diet C, Table II) yielded biomass comparable to the control but resulted in 19% mortality. In diets containing 4.5 mg% total fatty acids and no lecithin, increasing

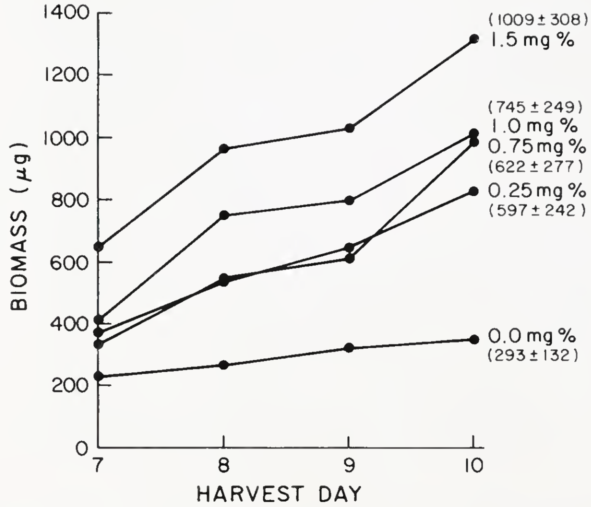

FIGURE 1. Effect of increasing dietary egg lecithin (70% pure) levels on the growth of *Moina macrocopa* fed an artificial diet. Control diet = 0.75 mg% lecithin. Liver infusion was present, soluble choline (as choline dihydrogen citrate from vitamin mix) = 0.14 mg%. Points for each day represent average biomass of five observations. (N) = average + SD biomass of all 20 observations for a particular diet. Standard error of the mean for any diet on any day = 72.8 μg. Average population mortality < 2%, except 14.3% for populations grown on diet with no lecithin.

soluble choline as choline dihydrogen citrate (diets D, E, F, Table II) further enhanced growth rates and reduced mortality. At 2 mg% choline dihydrogen citrate (0.7 mg% choline, diet F) population biomass was not significantly different ($P \leq 0.05$) than that of the pure egg lecithin diet (experiment II, Fig. 2) containing 0.069 mg% choline in the particulate form. The relative amounts of soluble and particulate sources of choline needed to achieve comparable growth rates indicate that a particulate source of choline is 10 times more efficient than a soluble source for these filter feeders.

Comparing the composite biomass data of the control diet with diet A, and that of diet C with diet D (Table II), suggests that the choline available from the vitamin mix additive (0.14 mg%) made no significant contribution to the population biomasses ($P \leq 0.01$).

Experiments IV and V

When *Moina* was fed diet A (Table III), which lacked both liver infusion and egg lecithin, growth was slow, and almost 40% of the population died. The amount of lecithin in the liver infusion was negligible (G. Holz, Suny, Upstate Medical Center, personal communication), but quantitative analysis showed that choline was 1.1% of the dry weight. A 40 mg% addition of liver infusion, therefore, provides 0.44 mg% choline, three times the level of soluble choline introduced via the vitamin mix of the control diet.

The experimental diets in which liver infusion was substituted with particulate choline, soluble choline, or a combination of the two demonstrated a growth response to choline. When 0.5 mg% soy lecithin was used as a particulate source of choline in a diet containing 3.0 mg% total fatty acids, and the amount of soluble

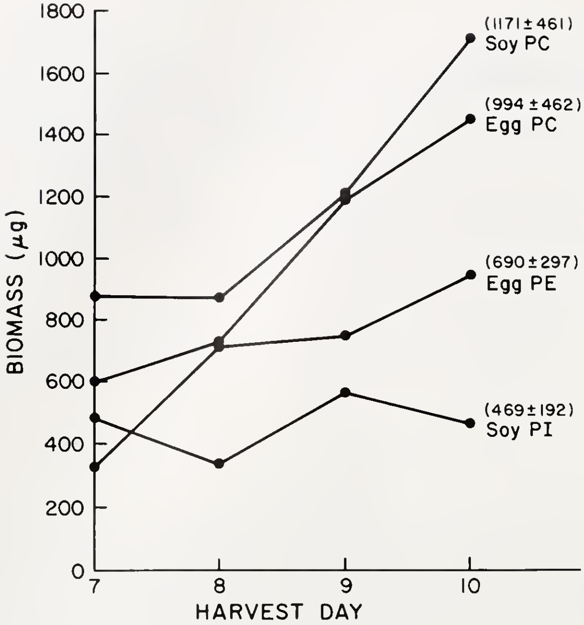

FIGURE 2. Effect of different pure phospholipids on growth of populations of *Moina macrocopa* fed an artificial diet. Phospholipid concentration was constant at 0.5 mg%. Liver infusion was present, soluble choline (as choline dihydrogen citrate in vitamin mix) = 0.14 mg%. Points for each day represent average biomass of 5 observations. (N) = average + SD biomass of all 20 observations for a particular diet. Standard error of the mean for any diet on any day = 112.95 μg. Average population mortality < 2%, except 12.8% for populations grown on diet containing PI.

choline was increased from 0.14 mg% (diet B) to 0.7 mg% (diet C), more animals survived, and biomass increased to 83.3% of that from a similar diet containing 40 mg% liver infusion and 0.14 mg% soluble choline (experiment II, soy PC, Fig. 2). Similar growth and survival occurred on diets in which the sole source of choline was soluble (1.40 mg%, diet D) or particulate (0.138 mg%, diet E). However, growth was significantly reduced from that of diets D and E ($P \leq 0.01$) by further increases in the concentration of total dietary choline, in either soluble form (2.00 mg%, diet F) or in combination (1.75 mg% soluble, 0.069 mg% particulate, diet G) (Table III). Figure 3 shows the relationship of total dietary choline (mg%) to the composite biomass of *Moina* populations grown on those diets that contained no liver infusion. Total dietary choline levels were standardized and expressed as particulate sources. Soluble choline amounts were converted to their particulate equivalent by assuming a previously described particulate-soluble nutrient source efficiency of 10 to 1. The fatty acid concentrations of these diets varied slightly from 2.5–4.5 mg%, but the growth response to choline is apparent.

Experimental diets that yielded composite biomass values that were less than 500 μg were considered nutritionally inferior. Often, the original female inoculant or her first clutch died, and many additional populations had to be started to attain some representative biomass data. This procedure introduced an element of artificial selection. We assume that under normal conditions these low biomass diets would not support continuous growth and reproduction. Population growth comparisons did not involve these nutritionally inadequate diets.

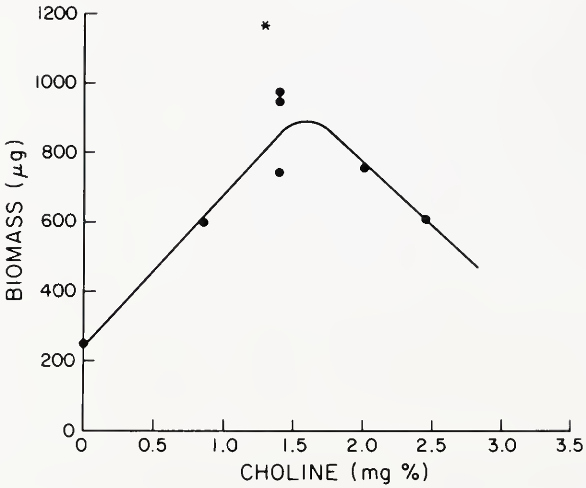

FIGURE 3. Relationship between the level of dietary choline and the average biomass of harvested populations of *Moina macrocopa* grown on artificial diets. Liver infusion was absent. Each point represents an average of 20 observations. * = average biomass of populations grown on soy PC diet (Experiment II).

DISCUSSION

Choline clearly is a required nutrient for growth and survival of the microcrustacean *Moina macrocopa*. In our artificial diets, this nutrient made its major contribution via the lecithin in the particulate portion of the biphasic media. Dietary lecithin, as a source of lipid, probably also contributes to increased growth rates. D'Abramo (1979) found that increasing dietary fatty acids from 3.0 to 4.5 mg% enhances growth of *Moina macrocopa*. Our observation of 10-fold greater effectiveness of a particulate rather than a soluble source of choline is lower than the 60-fold greater effectiveness of particulates estimated by Provasoli and D'Agostino (1969) for brine shrimp, *Artemia salina*, grown on artificial media. Provasoli and D'Agostino, however, based their estimates on growth with soluble amino acids versus particulate egg albumin. In such a situation, the inefficiency of soluble nutrient sources may be compounded by the crustacean's inability to utilize protein as singular amino acids rather than as polypeptides.

The population growth achieved by replacing the egg lecithin of the control diet with pure egg PE revealed that *Moina macrocopa* can efficiently synthesize choline via methylation of ethanolamine. This biosynthetic pathway is also described for the lobster *Homarus americanus* (Shieh, 1969). Use of this pathway alone, however, reduces population growth of *Moina* by 30%.

The greater nutritional value of pure soy PC versus pure egg PC was probably related to the different constituent fatty acids of the two ingredients. The qualitative effect of dietary fatty acids on the productivity of *Moina* has been discussed previously (D'Abramo, 1979). The poor quality of the diet containing pure soy phosphatidylinositol indicated that the requirement for lecithin is not a phospholipid requirement *per se*.

From the results obtained in experiment IV and the demonstrated increased effectiveness of particulate choline (10×), we estimate that the choline requirement for *Moina macrocopa* is 750–850 mg/100 g of particulate diet. Although choline has been shown to be a general requirement for insects, this is the first report of such a requirement for a microcrustacean. The choline requirement of several insects ranges from 150–900 mg/100 g of diet (Dadd, 1970). The quantitative choline requirement reported here for *Moina* is at the upper level of this range and may be partially attributed to accelerated growth rates associated with the warm (26°C) culture temperatures. Our value is considerably higher than the reported choline requirement of 45 mg/100 g for the prawn *Penaeus japonicus* (Kanazawa *et al.*, 1976). In contrast, another dietary study conducted with this prawn but for a longer time period (84 days vs. 40 days), demonstrates no choline requirement (Deshimaru and Kuroki, 1979). Research with the lobster *Homarus americanus* suggests that choline is a required nutrient (Bilinski, 1962).

Our results provide an explanation of the essentiality of the liver infusion in the artifical diet first formulated for *Moina macrocopa* by Conklin and Provasoli (1977): The infusion contributed approximately 0.07 mg% of the essential nutrient choline in the particulate form. Murphy and Davidoff (1972) observed that adding liver infusion at 70 mg% to a culture medium for *Moina macrocopa* reduces the time to production of the first clutch and increases the number of young per female. Most likely, their results principally reflect the effects of increased choline, since the amount of liver infusion added equals 0.77 mg% soluble choline, a 54% increase over the amount contained in the original medium (0.5 mg%).

The ability to replace the liver infusion component of the artificial diet with choline results in a completely defined medium for continuous culture of *Moina macrocopa*. Such substitution, however, was not complete because the highest composite biomass attained on a diet without liver infusion was less than that of a similar diet containing liver infusion. Although the liver infusion contained 14% neutral lipid, previous experiments (D'Abramo, 1979) show that defatted liver infusion is equally effective. The possible contribution of other water soluble vitamins such as riboflavin and folic acid remains to be investigated.

The present study shows that the absence or presence of a suboptimal amount of a micronutrient can significantly affect the survival, growth, and reproduction of *Moina macrocopa*. This implies that this culture technique could be used effectively for bioassays.

Our results also emphasize the possible shortcomings involved in estimating productivity of herbivorous zooplankton populations in terms of calorie, chlorophyll, or carbon content of coexisting phytoplankton populations. Moreover, the population dynamics of a zooplankton community may not only be regulated by factors such as temperature and predation. For example, competitive interactions may be based on the availability of a particular micronutrient and the ability of a species to store or use alternative biosynthetic pathways for production of that micronutrient. Indeed, seasonal succession of zooplankton species may be partially determined by their differing nutritional requirements.

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LITERATURE CITED

Bilinski, E. 1962. Biosynthesis of trimethylammonium compounds in aquatic animals. III. Choline metabolism in marine crustacea. *J. Fish. Res. Bd. Can.* **19:** 505–510.

Conklin, D. E., L. R. D'Abramo, C. E. Bordner, and N. A. Baum. 1980. A successful purified diet for the culture of juvenile lobsters: the effect of lecithin. *Aquaculture* **21:** 243–249.

Conklin, D. E., and L. Provasoli. 1977. Nutritional requirements of the water flea, *Moina macrocopa. Biol. Bull.* **152:** 337–350.

D'Abramo, L. R. 1979. Dietary fatty acid and temperature effects on the productivity of the Cladoceran, *Moina macrocopa. Biol. Bull.* **157:** 234–248.

D'Abramo, L. R. 1980. Ingestion rate decrease as a stimulus for sexuality in populations of the Cladoceran, *Moina macrocopa. Limnol. Oceanogr.* **25(3):** 422–429.

D'Abramo, L. R., C. E. Bordner, D. E. Conklin, and N. A. Baum. 1981. Essentiality of dietary phosphatidylcholine for the survival of juvenile lobsters. *J. Nutr.* **111:** 63–69.

Dadd, R. H. 1970. Arthropod Nutrition. Pp. 35–95 in M. Florkin and B. T. Scheer, Eds., *Chemical zoology*, Vol. V. Academic Press, New York.

Deshimaru, O., and K. Kuroki. 1979. Requirement of prawn for dietary thiamin, pyridoxine and choline chloride. *Bull. Jpn. Soc. Sci. Fish.* **45:** 363–367.

Kanazawa, A., S. Teshima, and N. Tanaka. 1976. Nutritional requirements of the prawn. V. Requirements for choline and inositol. *Mem. Fac. Fish. Kagoshima Univ.* **25(1):** 47–51.

Kanazawa, A., S. Teshima, S. Tokiwa, M. Endo, and F. Abdel Razek. 1979. Effects of short-necked clam phospholipids on the growth of the prawn. *Bull. Jpn. Soc. Sci. Fish.* **45(8):** 961–965.

Lester, R., M. C. Carey, J. M. Little, L. A. Cooperstein, and S. R. Dowd. 1975. Crustacean intestinal detergent promotes sterol solubilization. *Science* **189:** 1098–1100.

Lim, F., and E. D. Schall. 1964. Determination of choline in feeds. *J. Assoc. Off. Agric. Chem.* **47(3):** 501–503.

Murphy, J. S., and M. Davidoff. 1972. The result of improved nutrition on the Lansing effect in *Moina macrocopa. Biol. Bull.* **142:** 302–309.

Provasoli, L., and A. S. D'Agostino. 1969. Development of artificial media for *Artemia salina. Biol. Bull.* **136:** 434–453.

Shieh, M. S. 1969. The biosynthesis of phospholipids in the lobster, *Homarus americanus. Comp. Biochem. Physiol.* **30:** 679–684.

Zar, J. H. 1974. *Biostatistical analysis.* Prentice-Hall Inc., Englewood Cliffs, N. J. 620 pp.

Reference: *Biol. Bull.* **161:** 366–370. (December, 1981)

TENTACULAR NEMATOCYTE DISCHARGE AND "SELF-RECOGNITION" IN *ANTHOPLEURA ELEGANTISSIMA* BRANDT

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ABSTRACT

In the clonal anemone *Anthopleura elegantissima* isolated mucus from both clonemates and non-clonemates fails to elicit discharge of tentacular nematocytes. While direct contact between clonemates fails to elicit significant discharge, similar contact between non-clonemates does so.

Isolated mucus from a number of xenogeneic Zoantharia elicits discharge, and the reciprocal tests are positive. However, while mucus from the congeneric *A. xanthogrammica* fails to elicit discharge in *A. elegantissima*, the latter's mucus effects significant discharge in *A. xanthogrammica.*

The relation of these and other studies to our understanding of the physiology of nematocyte discharge is discussed.

INTRODUCTION

The anemone *Anthopleura elegantissima* is a common inhabitant of the intertidal zone on the West Coast of North America. It occurs in two forms, one solitary and one aggregating (Hand, 1955; Francis, 1979). Francis (1973a) showed that assemblages of the latter consist of clonal animals produced by longitudinal fission. She further (Francis, 1973b) showed that tentacular contact between individuals of different clones (non-clonemates) results in rapid withdrawal of those tentacles involved, often of nearby tentacles and even of the whole tentacular crown. Repeated contact between non-clonemates eventually elicits elaborate agonistic behavior, producing anemone-free zones between competing clones. In contrast, individuals of the same clone associate closely with each other in dense aggregations, and clonemate tentacles interlace without apparent interaction.

These anemones possess a mucus coat. One may ask whether this mucus is involved in distinguishing self from non-self. Lubbock (1979) investigated the mucus antigenicity of a number of anemone species and found no difference within species, but marked difference between species. He suggested that anemone mucus may be species-specific and that it may be one factor used in recognition. We have used the level of tentacular nematocyte discharge as the response criterion to investigate the role of mucus in self-recognition.

MATERIALS AND METHODS

All experiments were conducted in the Marine Laboratory of the University of California, Santa Barbara. Experimental animals consisted of the aggregating form of *A. elegantissima* from each of two phenotypically distinct clones, the solitary form of the same species, the congeneric *A. xanthogrammica*, and *Metri-*

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dium senile, Tealia lofotensis and *Corynactis californica.* All animals were kept in separate glass bowls in filtered, running seawater at 12°–13°C, under the normal day–night cycle of the laboratory (approx. 12 on and 12 off). The holding area and bowls were drained and rinsed frequently during the course of the experiments. All animals were acclimated to laboratory conditions for two weeks before the experiments began. At intervals they were fed small pieces of *Mytilus,* but never within 24 hours of experimentation.

An anemone whose responses were to be tested was held in a glass bowl supplied with oxygenated seawater. The bowl was placed under a dissecting microscope for observation. To avoid heat stress lighting was kept at the absolute minimum required for clear observation. Only anemones with attached pedal disks and expanded crowns were used. All experiments were conducted between 1200 and 1400.

The standard test for discharge was a modification of the technique of Davenport *et al.* (1961). A glass rod with adhered mucus was brought in contact with the tip of an arbitrarily chosen tentacle of the subject animal. If, upon moving the rod away, rod and tentacle adhered, the test was scored a positive; if not, a negative. Adhesion was assumed to result from nematocyte discharge. With this technique, variation of the intensity of contact is rendered insignificant *only* by large sample size. Accordingly, N = 100 in all test series. The rods used in the test were lightly etched with 48% hydrofluoric acid for better adhesion of mucus. Before use they were sterilized in 95% ethanol, flamed over a Bunsen burner and cooled. Occasionally after flaming residual carbon on a rod may elicit nematocyte discharge. As a control for this a rod to be used in a test was first brought in contact with a tentacle of the anemone about to be used as a donor of mucus. If the rod adhered, it was discarded. If it did not, it was then brought in contact with the oral disk or upper column of the donor until mucus had adhered to it. Test of the subject followed immediately.

In two test series (*e* and *f*, Table I) the tentacles of intact anemones which had settled on clean *Mytilus* shells (and therefore could be moved with little disturbance) were carefully brought into direct contact with tentacles of subjects. These were scored in the same manner as described above: if on moving the animals apart tentacles adhered, a positive was scored; if not, a negative.

RESULTS

Preliminary tests using solitary and clonal *A. elegantissima* gave no evidence of tentacular adhesion. Hence no further experiments were carried out with solitary animals.

TABLE I

Combined data from tests for tentacular adhesion to prepared glass rods and to intact animals in two phenotypically distinct clones of Anthopleura elegantissima. *In each series the N equals 100 tests for adhesion of individual, randomly selected tentacles. Positive indicates adhesion, negative non-adhesion.*

Stimuli	Positive	Negative
a. Clean rod (control)	1	99
b. Rod plus saliva (control)	93	7
c. Rod plus clonemate mucus	2	98
d. Rod plus non-clonemate mucus	4	96
e. Intact clonemate	1	99
f. Intact non-clonemate	39	61

TABLE II

Combined data from tests for tentacular adhesion in two clones of Anthopleura elegantissima *upon contact with glass rods carrying the mucus of four Pacific Zoantharia. N = 100.*

Mucus from:	Postive	Negative
A. xanthogrammica	6	94
Metridium senile	96	4
Tealia lofotensis	98	2
Corynactis californica	92	8

Experiment 1 was designed to test the hypothesis that the tentacular nematocytes of clonal *A. elegantissima* animals respond differently to the mucus of clonemates and non-clonemates. One series of tests was conducted each day for six days. Four of these series (*a–d*, Table I) involved the glass rod technique; in two series, *e* and *f*, animals were brought into direct contact. Each series involved ten subject anemones (five from each of the two different clones). Tests of a single, randomly selected tentacle of each animal were followed by a two-minute rest period. This was repeated ten times, giving a total of 100 tests per series.

Experimental protocol and results, as seen in Table I, clearly indicate that no significant discharge takes place in response to isolated mucus from either clonemate or non-clonemate. Thus, the hypothesis is untenable. However, the last two series showed that direct contact with an intact non-clonemate effects significant discharge, when such contact with a clonemate does not.

Experiment 2 tested the hypothesis that the nematocytes of *A. elegantissima* respond differently to the mucus of its congeneric relative *A. xanthogrammica* than they do to the mucus of more distantly related Zoantharia. Table II supports the hypothesis and indicates that while contact with *A. xanthogrammica* mucus elicits little discharge, heavy discharge occurs upon contact with the mucus of three non-congeneric Zoantharia, *Metridium senile, Tealia lofotensis* and *Corynactis californica.*

Experiment 3 tested the hypothesis that mucus from *A. elegantissima* will give the same spectrum of responses when tested against the tentacles of the four Zoantharia used as mucus donors in Experiment 2. The data of Table III disprove the hypothesis, for mucus from *A. elegantissima* causes heavy discharge in the congeneric *A. xanthogrammica*, whereas (Table II), mucus from *A. xanthogrammica* has little effect on *A. elegantissima.* In addition, while mucus from *A. elegantissima* effects little discharge in *Metridium senile*, mucus from *M. senile* causes heavy discharge in *A. elegantissima* (Table II).

TABLE III

Adhesion of individual tentacles upon contact with a control glass rod and with a rod carrying the mucus of Anthopleura elegantissima *in four Pacific Zoantharia. N = 100.*

Zoantharian	Clean glass rod (% adhering)	Rod with *A.elegantissima* mucus (% adhering)
A. xanthogrammica	0	92
M. senile	3	11
T. lofotensis	0	87
C. californica	11	76

DISCUSSION

The present study indicates that the system of tentacular nematocyte discharge in *Anthopleura elegantissima* discerns no difference in the mucus of clonemate and non-clonemate. Nevertheless, factors in the mucus may be involved in the recognition response that results in the avoidance of non-clonemates (Francis, 1973b). In our experiments, almost invariably upon the application of clonemate or non-clonemate mucus to the tentacle of a subject animal, a retraction occurred, often of neighboring tentacles and occasionally of the whole tentacular crown. A clean glass rod elicited no such reaction. This avoidance response is identical to that observed after contact between intact non-clonemates and briefly after initial contact between clonemates that have been isolated for a time. However, the agent in the mucus that elicits these overt intraspecific recognition responses does not appear to affect the discharge system of the tentacular nematocytes.

One of the most interesting facts to emerge from this study is that the discharge system of *A. elegantissima* treats the mucus of the congeneric *A. xanthogrammica* as self, while the converse is not true: the *A. xanthogrammica* system treats the mucus of *A. elegantissima* as non-self. The mucus of *A. elegantissima* must contain substances which trigger discharge in non-congeneric forms as seen in Table II. In some way the discharge system of *A. elegantissima* must be "buffered" against the effector-substance(s) present in its own mucus and that of *A. xanthogrammica*.

Active and passive models can explain these results. The passive model assumes that if an agent is applied to a tentacle and no discharge occurs, then either no effector-substance is in the agent or if one is, it is simply ineffective in lowering the threshold of the specific discharge system being tested. According to this model the effector-substance in the mucus of *A. elegantissima* that lowers the threshold of discharge in other Zoantharia is simply without effect on the animal's own system.

The active model (Schlichter, 1976) assumes that Zoantharia mucus contains an inhibitory agent which "protects" the animal's own discharge system against the effector-substances present in it. Although this model assumes a high level of species-specificity, we find it more tenable than the passive one. It accords with the suggestion of Lentz and Barnett (1962) that in *Hydra* discharge-effector substances are enzyme substrates which react with strategically placed enzymes on the nematocyte or cnidocil to effect discharge. These workers found that various organic phosphates augment nematocyte discharge and that discharge to the phosphates is inhibited by known enzymatic inhibitors. Such a system provides opportunities for the diversity in specificity which has been shown to exist in many experiments to date.

A recent study by Lubbock (1980) is relevant. Lubbock compared the specificity of the acrorhagial system in *A. elegantissima* with that of the tentacular system. He showed that neither group of nematocytes responds to excised syngeneic (clonemate) tissue, but both respond to allogeneic (non-clonemate) tissue. Tentacular nematocytes respond to a broad range of tissues from diverse taxa, while acrorhagial ones are far more specific and discharge only to certain allogeneic and xenogeneic (non-conspecific) Zoantharia. Our results concur that isolated mucus fails to elicit discharge in allogeneic animals (non-clonemates) as well as syngeneic ones, but that direct contact with an allogeneic individual (either the intact animal or tissue excised from it) effects discharge.

As yet we have no real knowledge of how recognition is mediated at the nematocyte, or of the stimulus-to-discharge chain. Some workers (Bigger, 1980) have considered whether antigen-antibody phenomena are involved in discharge. Hildemann *et al.* (1979) pointed out that the minimum criteria for an immunologic phenomenon have not been established in a nematocyte discharge system. One criterion is "inducible memory or selectively altered reactivity on secondary contact." In a brief experiment Lubbock (1980), using acrorhagial nematocyte discharge as the criterion, was unable to show that repeated aggressions against one particular clone specifically enhanced the aggressive response to that clone.

Many questions about the nematocyte discharge system remain unanswered. Although spatially limited, the system is highly sophisticated and is affected by agents working at different points. These points are unknown. We still do not understand how the effects of mechanical and chemical stimulation are coupled. Moreover, we know little or nothing about changes in discharge level over time, though that these changes occur is well established (Davenport *et al.*, 1961; Ross and Sutton, 1964; MacFarlane and Shelton, 1975).

LITERATURE CITED

Bigger, C. H., 1980. Interspecific and intraspecific acrorhagial aggressive behavior among sea anemones: a recognition of self and non-self. *Biol. Bull.* **159:** 117–134.

Davenport, D., D. M. Ross, and L. Sutton. 1961. The remote control of nematocyst discharge in the attachment of *Calliactis parasitica* to shells of hermit crabs. *Vie et Milieu* **12:** 197–209.

Francis, L. 1973a. Clone specific aggregation in the sea anemone *Anthopleura elegantissima. Biol. Bull.* **144:** 64–72.

Francis, L. 1973b. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related anemones. *Biol. Bull.* **144:** 73–92.

Francis, L. 1979. Contrast between solitary and clonal lifestyles in the sea anemone *Anthopleura elegantissima. Amer. Zool.* **19:** 669–681.

Hand, C. 1955. The sea anemones of central California, Part II. The endomyarian and mesomyarian anemones. *Wasmann J. Biol.* **13:** 37–99.

Hildemann, W. H., D. S. Linthicum, and D. C. Vann. 1979. Immunocompatibility reactions in corals (Coelenterata). Pp. 105–114 in W. H. Hildemann and A. A. Benedict, Eds., *Immunologic Physiology, Advances in Experimental Medicine and Biology,* vol. 64, Plenum Press, New York.

Lentz, T. L., and R. J. Barrnett. 1962. The effect of enzyme substrates and pharmacological agents on nematocyst discharge. *J. Exper. Zool.* **149:** 33–38.

Lubbock, R. 1979. Mucus antigenicity in sea anemones and corals. *Hydrobiol.* **66:** 3–6.

Lubbock, R. 1980. Clone-specific cellular recognition in a sea anemone. *Proc. Nat. Acad. Sci.* **77:** 6667–6669.

MacFarlane, I. D., and G. A. B. Shelton. 1975. The nature of the adhesion of tentacles to shells during shell-climbing in the sea anemone *Calliactis parasitica. J. Exper. Mar. Biol. Ecol.* **17:** 177–186.

Ross, D. M., and L. Sutton. 1964. Inhibition of the swimming response by food and of nematocyst discharge during swimming in the sea anemone *Stomphia coccinea. J. Exper. Biol.* **41:** 751–757.

Schlichter, D. 1976. Macromolecular mimicry: substances released by sea anemones and their role in the protection of anemone fishes. Pp. 433–441 in G. O. Mackie, Ed., *Coelenterate Ecology and Behavior.* Plenum Press, New York.

Reference: *Biol. Bull.* **161:** 371–381. (December, 1981)

THE EFFECTS OF INHIBITORS AFFECTING PROTEIN SYNTHESIS AND MEMBRANE ACTIVITY ON THE *CHLAMYDOMONAS REINHARDII* PHOTOTACTIC RHYTHM

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ABSTRACT

Cycloheximide and puromycin slowed the biological timing of the phototactic rhythm of *Chlamydomonas reinhardii.* However, actinomycin D, rifampicin, chloramphenicol, streptomycin, chloral hydrate, valinomycin, LiCl, ethanol, methanol, isopropanol, procaine, tetracaine, dibucaine, and sodium lauryl sulfate did not alter the period length.

A cycloheximide resistant mutant (*cyr*-1) was isolated. The mutation is recessive since diploids produced from *cyr*-1 and wild type are sensitive to cycloheximide. The phototactic rhythm of *cyr*-1, unlike that of the wild type, is not influenced by cycloheximide. This supports the view that the action of this drug in slowing the rhythm in the wild type is mediated by the inhibition of protein synthesis.

INTRODUCTION

The cellular and behavioral activities of many eucaryotic organisms are regulated by a biological clock that is separate from the activities it governs. This biological timing mechanism is adaptive because it allows organisms' activities to occur at the most propitious time, either of the day or relative to another rhythmic activity. Although many of the properties of this clock have been identified, an understanding of the molecular mechanism of the circadian clock still eludes us.

One method of attempting to decipher the mechanism of the clock has been to subject an organism to chemicals with known effects, hoping that they will alter the clockworks in a manner that will reveal some aspect of its machinery. If a drug reaches the clock and interferes with a process important to timekeeping, its sustained administration would be expected to result in a change in the period length of the observed rhythm. Because many substances inhibit cellular processes and are fatal when administered continuously, and because it is of interest to identify particular stages of the circadian cycle that are maximally sensitive to a substance, drugs are often added for only a short period. When chemicals are pulsed in this way, the investigator looks for a change in the phase of the rhythm.

The effects of protein synthesis inhibitors on several organisms and rhythms have been explored using these techniques. Two generalizations about the effectiveness of these inhibitors are beginning to emerge: inhibitors of protein synthesis on 70S ribosomes, such as streptomycin and chloramphenicol, are usually ineffective (Hastings, 1960; Karakashian and Hastings, 1962, 1963; Sweeney *et al.,* 1967; Enright, 1971; Mergenhagen and Schweiger, 1975a), but inhibitors of protein syn-

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Abbreviations: + mating type, mt+; − mating type, mt−; high salt-concentration medium, HSM.

[3] Some of these experiments were initially done by Anthony Carter as part of a Senior Honors thesis (unpublished) at Princeton University.

thesis on 80S ribosomes, such as cycloheximide, puromycin, and anisomycin, do affect the period or phase of some rhythms (Karakashian and Hastings, 1963; Brinkmann, 1971, 1973; Feldman, 1967; Mergenhagen and Schweiger, 1975b; Karakashian and Schweiger, 1976a, 1976b; Rothman and Strumwasser, 1976, 1977; Jacklet, 1977; Straley and Bruce, 1979; Walz and Sweeney, 1979; Dunlap *et al.*, 1980). Therefore, one might tentatively infer either that 80S protein synthesis is involved in the mechanism of the clock or that protein components of the clock must be periodically resynthesized.

The results of other experiments are consistent with the suggestion that membrane structure or function is important to circadian time-keeping. If this is true, then protein synthesis may affect the clock indirectly by influencing the supply of a protein involved in active transport (Sargent *et al.*, 1976). The evidence that membranes are actually involved in the generation of circadian oscillations is mainly circumstantial. Substances such as ethanol (Keller, 1960; Bünning and Baltes, 1962; Bünning and Moser, 1973; Brinkmann, 1974, 1976; Sweeney, 1974; Taylor *et al.*, 1979), valinomycin, an ionophore of K^+ (Bünning and Moser, 1972; Sweeney, 1974, 1976), and ions such as K^+ (Eskin, 1972; Bünning and Moser, 1973) and Li^+ (Engelmann, 1972, 1973; Engelmann *et al.*, 1974; Eskin, 1977) modify some rhythms and are also known to influence membranes and/or transport.

Genetics also has been used to probe the mechanism of the clock. Genetic analysis has isolated mutants with altered clock properties in *Drosophila* (Konopka and Benzer, 1971; Pittendrigh, 1974), *Neurospora* (Feldman and Wasser, 1971; Feldman *et al.*, 1973; and Feldman and Hoyle, 1974) and *Chlamydomonas* (Bruce, 1972, 1974). In addition, mutations altering some aspect of physiology have been studied to determine whether the mutations also affected the circadian clock (Feldman, *et al.*, 1979).

Combining the chemical manipulation and genetic approaches has been informative. Clock properties can be compared in wild type organisms sensitive to a particular chemical and in organisms carrying a mutation for resistance to that chemical. Nakashima *et al.* (1981) have shown that in two *Neurospora* mutants whose growth is resistant to cycloheximide, the conidiation rhythms also are unaffected by the drug. When Dieckman and Brody (1980) crossed a mutation of resistance to oligomycin into the band strain of *Neurospora*, the period length decreased from the 21.5 h characteristic for the band strain to 18–19 h.

The most useful picture of the pattern of effects of chemicals emerges when a whole spectrum of inhibitors is tested on the circadian system of a single organism. *Chlamydomonas* is a unicell that has a well defined circadian rhythm in phototaxis (Bruce, 1970). This study tested effects of a variety of chemicals on this rhythm to identify cellular processes that may be important to circadian timing in this organism.

Materials and Methods

Culture techniques

Chlamydomonas reinhardii strains, mt^+ and mt^- (Bruce, 1970), as well as the clock mutants per 1 mt−, per 2 mt−, and per 4 mt^+ (Bruce 1972, 1974), and a cycloheximide resistant mutant (described below) were used in these experiments. Experimental cultures were grown on a shaker table under continuous illumination from cool white fluorescent lamps (1500–3000 lux) at 22°C. The cultures were grown to a density of 1–2 × 10^6 cells/ml.

Phototactic assay

Phototactic rhythm assays were made as described previously (Bruce, 1970). Samples of the culture (1.5 ml) were placed in individual wells of plastic tissue-culture trays and the drugs were added to the final concentrations indicated at the beginning of each experimental test.

Cell counts

Samples of the culture were diluted 1:100 with 0.9% saline solution, and then cell counts were made with a Coulter counter. Unless otherwise specified, cultures were counted at 1 or 2 day intervals until high cell density limited growth.

Genetic analysis

The cycloheximide resistant mutant *cyr*-1 was isolated from mt− after exposure to the mutagen nitrosoguanadine (Bruce, 1972) and plating on 0.3 high salt-concentration medium (HSM) plates supplemented with 10 μg/ml of cycloheximide. Diploids of *cyr*-1 were constructed using arginine-requiring strains (Bruce and Bruce, 1978). They were confirmed to be diploids by crossing them with wt and recovering arginine-requiring progeny. Sensitivity or resistance to cycloheximide was determined by spot plating on plates supplemented with 10 μg/ml cycloheximide.

Chemicals

The following chemicals were tested on the phototactic rhythm:

Drug	Range
actinomycin (Merck, Sharpe, and Dohm)	0.25, 0.5, 1.0, 2.0, 4.0 μg/ml
rifampicin (CIBA)	0.25, 0.5, 1.0, 2.0, 4.0 μg/ml
chloramphenicol (Sigma)	2.75, 5.5, 11.25, 22.5, 45 μg/ml
streptomycin sulfate (Lily)	45.5, 60, 70 μg/ml
cycloheximide (Actidione) (Upjohn Co, Kalamazoo	0.05, 0.08, 0.1, 0.15, 0.2, 0.25, 0.5, 1, 4 μg/ml
puromycin dihydrochloride (Sigma)	1.3, 26, 130 μg/ml
valinomycin (Sigma)	0.09, 0.45, 0.83 μg/ml
LiCl (Fischer)	0.05, 0.09, 0.36, 0.45, 0.64 m*M*
procaine (Sigma)	0.33, 1.0, 3.3, 10 mg/ml
tetracaine (Sigma)	0.033, 0.1, 0.33, 1.0 mg/ml
dibucaine	0.0033, 0.01, 0.033, 0.1 mg/ml
sodium lauryl sulfate	0.0033, 0.01, 0.033, 0.1 mg/ml
ethanol	0.005, 0.18, 0.27%
methanol	0.1, 1.0, 2.0%
isopropanol	0.1, 1.0, 2.0%
chloral hydate	0.167, 0.5, 0.99, 1.67, 5.0 mg/ml

RESULTS

Inhibitors of transcription

Actinomycin D (added in ethanol) in concentrations up to 2 μg/ml allowed phototaxis to be expressed but had no effect on the period length of the rhythm. At the highest concentration, phototaxis was inhibited. Growth was inhibited in a dose-related manner at all drug concentrations. The period length of ethanol controls was the same as that of controls in untreated media.

Rifampicin did not alter the period length of the phototactic rhythm and had no reliable effect on the growth rate.

Inhibitors of translation

Chloramphenicol did not affect either the phototactic rhythm or growth rate.

Streptomycin had no effect on the period length of the rhythm but eliminated rhythmicity in some samples after approximately 4 cycles. Higher concentrations killed the cells.

Puromycin dihydrochloride at low concentrations (1.3 and 26 μg/ml) had no effect on the phototactic rhythm, but a concentration of 130 μg/ml increased the period length 8.3%, from 24 to 26 h (Fig. 1). The growth rate was inhibited at all concentrations, but at lower concentrations the inhibition was not immediate.

Cycloheximide's period lengthening effect on the per 2 mt− strain is shown in Figure 2. The effect on the rhythm was immediate and dose-related. The period lengthening effects on per 2 mt−, per 4 mt^+ and wt mt− are summarized in Figure 3. Although cycloheximide consistently lengthened the period in all the experiments, some differences between strains were noted in different experiments, and the drug's effect did not always persist indefinitely. For example, 0.15 and 0.25 μg/ml of cycloheximide lengthened the period of the per 1 mt− mutant for the initial 2–3 cycles after exposure, but the period eventually returned to the same length as controls (Fig. 4).

Cell counts taken over several days, but at 3 h intervals during the first day, indicate that in all samples treated with 0.1, 0.2, 1, and 4 μg/ml cycloheximide, growth was inhibited for the first 12 h of exposure. After the first 12 h, all the

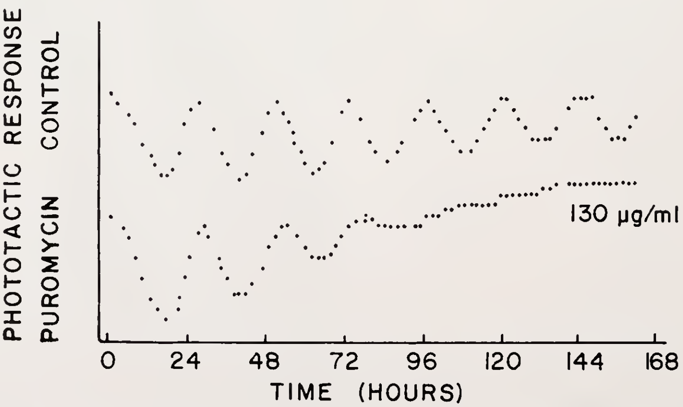

FIGURE 1. The rhythmic phototactic response of mt− strain of *Chlamydomonas reinhardii* and its modification by puromycin.

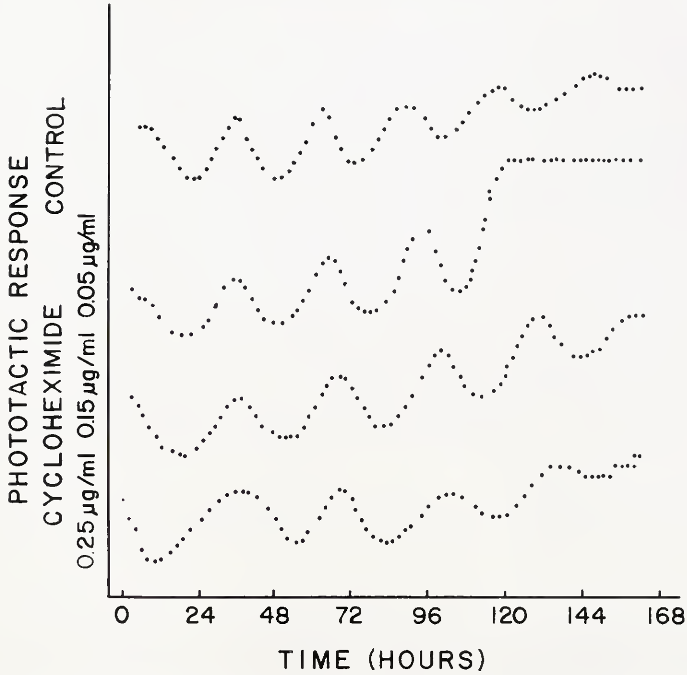

FIGURE 2. The rhythmic phototactic response of the mt− strain of *Chlamydomonas reinhardii* and its modification by cycloheximide.

period mutants treated with 0.1, or 0.2 µg/ml of cycloheximide had increased growth rates. At higher drug concentrations, the inhibition of growth lasted slightly longer. In cultures treated with 4 µg/ml, the cell growth that did occur ceased after the first 24 h. Cell numbers fell below the number counted on day 1 and cells were bleached.

Although cycloheximide lengthens the period of many mutants, this is not the case with the cycloheximide resistant mutant *cyr*-1, whose rhythm is unaffected by concentrations of cycloheximide which completely inhibit the response of the wild type. We have constructed diploids of *cyr*-1 and the wild type. Because of the way in which diploids are obtained in *Chlamydomonas*, they also contain arginine markers, in this case various combinations of arg 1, arg 2, and arg 7. All twelve diploids tested were sensitive to cycloheximide. The drug's effect on the period length of the diploid's rhythm was similar to that of the wild types. The average period lengthening effect of 0.5 µg/ml was 2.3 h (10 diploids) and of 1.5 µg/ml, 3.1 h (9 diploids).

Chloral hydrate did not affect the rhythms of mt− or the per 2 mutant at drug concentrations up to 0.99 mg/ml. The mt− strain was tested at 17° and 23°C.

"Membrane-active" chemicals

The alcohols methanol, ethanol, and isopropanol did not influence the phototactic rhythm at the concentrations tested.

Valinomycin and valinomycin (0.45 µg/ml) with additional K^+ ions (4.55 m*M* KCl) also did not alter the period length of the rhythm.

Li^+ ions in the form of LiCl did not alter the rhythm.

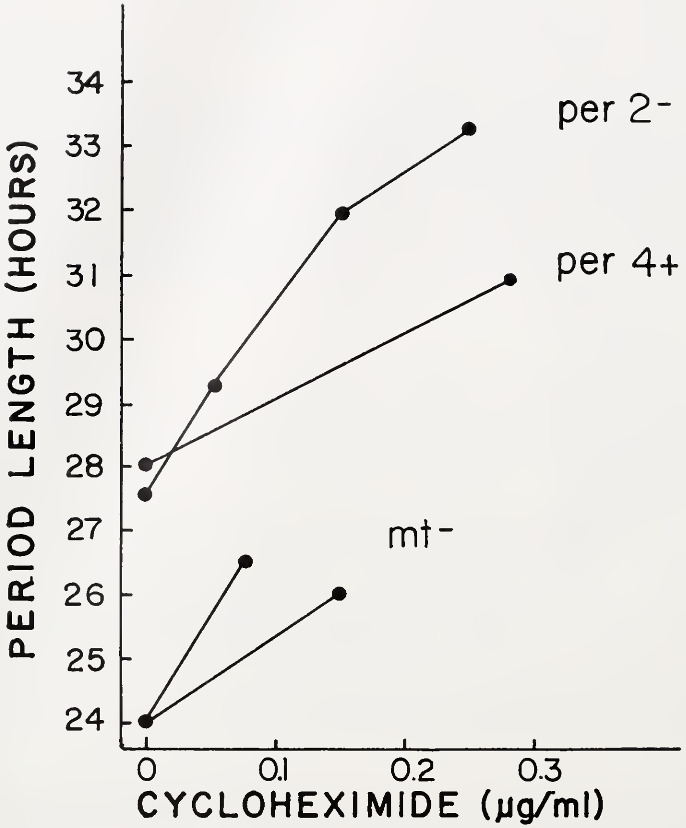

FIGURE 3. Period lengthening effects of cycloheximide on the wild type mt− and the mutants per 2 mt− and per 4 mt$^+$.

The local anesthetics, procaine, tetracaine and dibucaine had no effect on the rhythm when tested at concentrations 1/3, 1/10 and 1/30 times the minimum values that inhibited phototaxis.

DISCUSSION

Inhibitors of transcription and translation

Our survey of the effects of inhibitors of transcription and translation on the *Chlamydomonas* rhythm support the generalization from other clock systems that the aspect of gene expression important to the timing of circadian rhythms is the translation of proteins on the 80S ribosomes. This statement is based on the increase in the period length of the rhythm that results from sustained administration of cycloheximide and puromycin, chemicals that inhibit translation on the 80S ribosomes. Antibiotics that inhibit transcription, such as AcD and rifampicin, and those that inhibit translation on the 70S ribosomes, such as streptomycin and chloramphenicol, did not appear to slow the biological timing process. These data are consistent with the generalizations mentioned earlier that translation of proteins on the 80S but not the 70S ribosomes is essential to the timing of circadian rhythms.

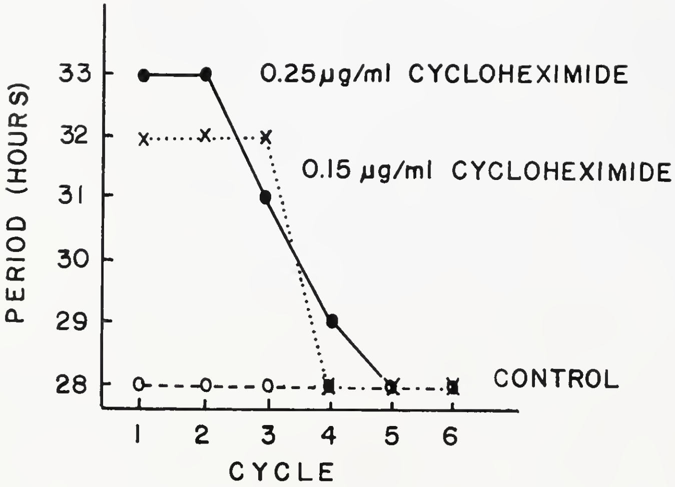

FIGURE 4. The effect of cycloheximide on the period length of the phototactic rhythm of the per 1 mt−.

To test whether particular chemicals did enter cells we measured the effect of the inhibitors on growth. The reason that AcD did not affect phototactic rhythm probably was not its inability to enter the cells, since concentrations that had no effect on the timing of the rhythm did inhibit cell growth. AcD is destroyed by light, which is essential to assay the phototactic response. However, the growth rate was inhibited by AcD in cultures grown in continuous light, so destruction of the inhibitor by light cannot explain the lack of effect on the circadian timing. On the other hand, rifampicin did not inhibit cell growth in cultures grown in continuous light or continuous darkness, so the question of whether it entered the cells remains.

Chloramphenicol also did not influence growth rate or the phototactic rhythm in these experiments. Jacobson *et al.* (1964) demonstrated that $3 \times 10^{-4} M$ chloramphenicol had an antimitotic effect on *Chlamydomonas reinhardii*, indicating the chemical can enter the cells. However, the drug concentrations used in the present experiment are below those used by Jacobson *et al.*, and therefore, may have been too low to diffuse to its sites of action, the mitochondria and chloroplasts. The second inhibitor of translation on the 70S ribosomes tried in this experiment, streptomycin, also did not alter the period length of the phototactic rhythm. We did not determine its effect on growth rate. However, the appearance of arrhythmicity in some of the samples treated with 45.5 μg/ml streptomycin, and the fact that higher drug concentrations killed the cells, suggest that this inhibitor was able to enter the cells, and that its failure to affect the timing of the clock is evidence that translation of the 70S ribosomes is not essential to the timing mechanism.

Both puromycin and cycloheximide, the inhibitors of translation on the 80S ribosomes tested in these experiments, slowed biological timing. Cycloheximide was effective at low concentrations, whereas the relatively high concentration of puromycin required to affect growth, as well as the rhythms, probably reflects the cells' relative impermeability to this drug.

Cycloheximide was previously shown to lengthen the period of the *Chlamydomonas* sticking rhythm (Straley and Bruce, 1979). In some of their experiments, as well as in a few of the present experiments on phototactic rhythm, the clock was only affected by cycloheximide for 2–3 cycles before the rhythm began to return to the period length of the controls (Fig. 4). We do not know why this occurs. However, since the drug's effect on the growth rate of the cells was similar, it may reflect some change in the cells' ability to deal with cycloheximide, perhaps by an inducible enzyme that degrades cycloheximide or by changes in permeability.

Since the diploids were created from the combination of a strain resistant to cycloheximide and a wild type strain, the fact that all diploids were as sensitive to cycloheximide as the wild type indicates that the mutation for resistance (*cyr*-1) is recessive. The fact that cycloheximide does not alter the period of the resistant mutant *cyr*-1, but lengthens the period of the recessive diploid, suggests that this drug affects the rhythm because of its inhibition of protein synthesis, rather than via some secondary effect of cycloheximide. Our observations on *Chlamydomonas* are in agreement with those of Nakashima *et al.* (1981) with *Neurospora.* These workers found that the conidiation rhythms of two cycloheximide resistant mutants are unaffected by the drug. The site of cycloheximide resistance in the *Neurospora* mutants is known to be protein synthesis on 80S ribosomes.

Drugs commonly have more than one effect on living cells. Therefore, one can never be sure that a chemical that affects the clock is exerting its influence via its primary mode of action. One way to increase the certainty that a drug is acting through its assumed mode of action is to test other chemicals that affect the same process. In this study, more than one inhibitor of transcription, of translation on 70S ribosomes and of translation on 80S ribosomes, were tested for their effectiveness on the phototactic rhythm. The effects of inhibitors with similar primary modes of action were always the same.

Although translation on the 80S ribosomes seems essential to the functioning of the circadian clock controlling phototactic rhythm in *Chlamydomonas*, we do not know whether this translation is directly involved as a part of the timing process, or indirectly involved by influencing some other cellular component.

The membrane-active chemicals

Many workers have suggested that membrane structure and/or function may be the basis for circadian timekeeping (see Sweeney, 1976 for a review of the evidence). We have tried a variety of chemicals believed to have affected the structure and or function of membranes under the conditions of our experiments. None altered the period of the phototactic rhythm.

One approach in the attempt at altering membrane structure or function was the continuous administration of local anesthetics (procaine, tetracaine, and dibucaine) known to act at the membrane level, and of a detergent, sodium lauryl sulfate. None of these treatments altered the period length of the rhythm.

Ethanol alters membrane fluidity (Chin and Goldstein, 1976) and active transport (Kalant, 1971). Both these aspects of the membrane are important in the timing mechanism of the clock, according to the membrane hypothesis of Njus *et al.* (1974), which proposed that the clock's timing mechanism is a system of feedback between the concentration of an ion on one side of the membrane and the active transport of that ion across the membrane. Although other workers have found that ethanol may alter biological timing, its effect is not consistent on all systems. It increases the period length of the *Phaseolus* sleep movement rhythm (Keller, 1960) and the *Euglena* motility rhythm (Brinkmann, 1974, 1976) but it shortens the period of the petal movement rhythm of *Kalanchoe* (Kastenmeier, *et al.*, 1977) and the glow rhythm of *Gonyaulax* (Taylor *et al.*, 1979). Our failure to find any effect on the period length of *Chlamydomonas* is one more inconsistent piece in the puzzle.

Brinkmann (1976) has noted that the effect of alcohols on biological timing in *Euglena* and in *Gonyaulax* (Sweeney, 1976) decreases with increasing carbon chain length. On the other hand, the effect of an alcohol on a membrane increases with carbon chain length. He suggested that the effect of alcohols on the clock may be through metabolism. In our experiments, methanol, ethanol, and isopropanol did not change the period of length.

Because ion transport has been suggested as an important component of the clock mechanism (Njus *et al.*, 1974) many workers have tried to alter biological timing with valinomycin, an ionophore of K^+ ions, or with the ions K^+ and Li^+. Phase changes from pulses of valinomycin (Sweeney, 1974, 1976) and K^+ (Eskin, 1972; Bünning and Moser, 1973) and period lengthening from Li^+ (Engelmann, 1972, 1973) have been reported. In contrast, however, the *Chlamydomonas* clock was unaltered by these chemicals.

Although planned pulsing experiments may elucidate points in the cycle that are affected by "membrane-active" chemicals, our results do not support the view that the membrane is important for functioning of the *Chlamydomonas* clock.

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LITERATURE CITED

Brinkmann, K. 1971. Metabolic control of temperature compensation in the circadian rhythm of *Euglena gracilis.* Pp. 567–593 In: *Biochronometry*, M. Menaker, Ed. National Academy of Sciences, Washington, D. C.

Brinkmann, K. 1973. The role of actidion in the temperature jump response of the circadian rhythm in *Euglena gracilis.* Pp. 523–529 In: *Biological and Biochemical Oscillators*, B. Chance, Ed. Academic Press, New York.

Brinkmann, K. 1974. The effect of ethanol on the metabolism and circadian rhythms of *Euglena gracilis. J. Interdiscipl. Cycle Res.* **5:** 186.

Brinkmann, K. 1976. The influence of alcohols on the circadian rhythm and metabolism of *Euglena gracilis. J. Interdiscipl. Cycle Res.* **7:** 149–170.

Bruce, V. G. 1970. The biological clock in *Chlamydomonas reinhardi. J. Protozool.* **17:** 328–334.

Bruce, V. G. 1972. Mutants of the biological clock in *Chlamydomonas reinhardi.* Genetics **70:** 536–548.

Bruce, V. G. 1974. Recombinants between clock mutants of *Chlamydomonas reinhardi. Genetics* **77:** 221–230.

Bruce, V. G., and N. C. Bruce. 1978. Diploids of clock mutants of *Chlamydomonas reinhardi. Genetics* **89:** 225–233.

Bünning, E., and J. Baltes. 1962. Wirkung von Athylalkohol auf die physiologische Uhr. *Naturwissenschaften* **49:** 19–20.

Bünning, E., and I. Moser. 1972. Influence of valinomycin on circadian leaf movements in *Phaseolus. Proc. Nat. Acad. Sci.* U. S. A. **69:** 2732–2733.

Bünning, E., and I. Moser. 1973. Light-induced phase shifts of the circadian leaf movements of *Phaseolus*: comparison with the effects of potassium and ethyl alcohol. *Proc. Nat. Acad. Sci. U. S. A.* **70:** 3387–3389.

Chin, J. H., and D. B. Goldstein. 1976. Increased membrane fluidity caused by ethanol in spin labelled mouse membranes. *Fed. Proc.* **35:** 707.

Dieckmann, C., and S. Brody. 1980. Circadian rhythms in *Neurospora crassa*: oligomycin-resistant mutations affect periodicity. *Science* **207:** 896–898.

Dunlap, J. C., W. Taylor, and J. W. Hastings. 1980. The effect of protein synthesis inhibitors on the *Gonyaulax* clock. I. Phase-shifting effects of cycloheximide. *J. Comp. Physiol.* **138:** 1–8.

Engelmann, W. 1972. Lithium slows down the *Kalanchoe* clock. *Z. Naturforsch.* **27b:** 477.

Engelmann, W. 1973. A slowing down of circadian rhythms by lithium ions. *Z. Naturforsch.* **28c:** 733–736.

Engelmann, W., A. Mauer, M. Muhlbach, and M. Johnson. 1974. Action of lithium ions and heavy water in slowing circadian rhythms in petal movement in *Kalanchoe. J. Interdiscipl. Cycle Res.* **5:** 199–205.

Enright, J. T. 1971. The internal clock of drunken isopods. *Z. Vgl. Physiol.* **72:** 1–16.

Eskin, A. 1972. Phase shifting a circadian rhythm in the eye of *Aplysia* by high potassium pulses. *J. Comp. Physiol.* **80:** 353–376.

Eskin, A. 1977. Neurophysiological mechanisms involved in the photoentrainment of circadian rhythm from the *Aplysia* eye. *J. Neurobiol.* **8:** 273–299.

Feldman, J. 1967. Lengthening the period of a biological clock in *Euglena* by cycloheximide an inhibitor of protein synthesis. *Proc. Nat. Acad. Sci. U. S. A.* **57:** 1080–1087.

Feldman, J. F., and M. N. Hoyle. 1974. A direct comparison between circadian and non-circadian rhythms in *Neurospora. Plant Physiol.* **53:** 928–930.

Feldman, J. F., and N. Wasser. 1971. New mutations affecting circadian rhythmicity in *Neurospora.* Pp. 652–656. In: *Biochronometry.* M. Menaker, Ed. Washington, D. C. Nat. Acad. Sci.

Feldman, J. F., M. N. Hoyle, and J. Shelgren. 1973. Circadian Clock mutants of *Neurospora crassa*; Genetic and Physiological characteristics (Abstract), *Genetics* **74:** S77–78.

Feldman, J. F., G. Gardner, and R. Denison. 1979. Genetic analysis of the circadian clock of *Neurospora.* Pp. 57–66. In: M. Suda, O. Hayaishi, and H. Nagagawa, Eds., *Biological rhythms and their central mechanism,* Elsevier/North Holland Biomedical Press.

Hastings, J. W. 1960. Biochemical aspects of rhythms: phase shifting by chemicals. *Cold Spring Harbor Symp. Quant. Biol.* **25:** 131–143.

Jacklet, J. W. 1977. Neuronal circadian rhythm: phase shifting by a protein synthesis inhibitor. *Science* **198:** 69–71.

Jacobson, B. S., R. J. Salmon, and L. L. Lansky. 1964. Antimitotic effects of chloramphenicol and other inhibitory agents on *Chlamydomonas. Exp. Cell Res.* **36:** 1–13.

Kalant, H. 1971. Absorption, diffusion, distribution and elimination of ethanol: effects of biological membranes. Pp. 1–61. In: *Biochemistry,* B. Kissin and H. Begleiter, Eds. Plenum Press, N. Y.

Karakashian, M. W., and J. W. Hastings. 1962. The inhibition of a biological clock by actinomycin D. *Proc. Nat. Acad. Sci. U. S. A.* **48:** 2130–2137.

Karakashian, M. W., and J. W. Hastings. 1963. The effects of inhibitors of macromolecular biosynthesis upon the persistent rhythm in luminescence in *Gonyaulax. J. Gen. Physiol.* **47:** 1–12.

Karakashian, M. W., and H-G. Schweiger, 1976a. Evidence for a cycloheximide-sensitive component in the biological clock of *Acetabularia. Exp. Cell Res.* **98:** 303–312.

Karakashian, M. W., and H-G. Schweiger, 1976b. Temperature dependence of the cycloheximide-sensitive phase of the circadian cycle in *Acetabularia mediterranea. Proc. Nat. Acad. Sci. U. S. A.,* **73:** 3216–3219.

Kastenmeier, B., V. Reich, and W. Engelmann. 1977. Effect of alcohols on the circadian petal movement rhythm of *Kalanchoe* and the rhythmic movement of *Desmodium. Chronobiologia* **4:** 122.

Keller, S. 1960. Uber die Wirkung chemischer Faktoren und die tagesperiodischen Blattbewegungen von *Phaseolus multifloris. Z. Bot.* **48:** 32–57.

Konopka, R. J. and S. Benzer. 1971. Clock mutants of *Drosophila melanogaster. Proc. Nat. Acad. Sci. U. S. A.* **68:** 2112–2116.

Mergenhagen, D., and H-G. Schweiger. 1975a. Circadian rhythm of oxygen evolution in cell fragments of *Acetabularia mediterranea. Exp. Cell Res.* **92:** 127–130.

Mergenhagen, D., and H-G. Schweiger. 1975b. The effect of different inhibitors of transcription and translation on the expression and control of circadian rhythms in individual cells of *Acetabularia. Exp. Cell Res.* **94:** 321–326.

Nakashima, H., J. Perlman, and J. F. Feldman. 1981. Genetic evidence that protein synthesis is required for the circadian clock of *Neurospora. Science* (in press).

Njus, D., F. M. Sulzman, and J. W. Hastings. 1974. Membrane model for the circadian clock. *Nature* **248:** 116–120.

Pittendrigh, C. S. 1974. Circadian Oscillations in Cells and the Circadian Organization of Multicellular System. Pp. 437–458. In: *The Neurosciences—Third Study Program*, Schmitt, F. O. and F. G. Warden, Eds. Cambridge, Mass. MIT Press.

Rothman, B. S., and F. Strumwasser. 1976. Phase shifting the rhythm in neuronal activity in the isolated *Aplysia* eye with puromycin and cycloheximide. *J. Gen. Physiol.* **68:** 359–384.

Rothman, B. S., and F. Strumwasser. 1977. Manipulations of a neuronal oscillator with inhibitors of macromolecular synthesis. *Fed. Proc.* **36:** 2050–2066.

Sargent, M. L., I. E. Ashkenazi, E. M. Bradbury, V. G. Bruce, C. F. Ehret, J. F. Feldman, M. W., Karakashian, R. J. Konopka, D. Mergenhagen, G. P. Schutz, H.-G. Schweiger, and T. E. Vanden Driessche. 1976. The Role of Genes and Their Expression. In: *The Molecular Basis of Circadian Rhythms*, Pp. 295–310. J. W. Hastings, and H.-G. Schweiger, Eds. Germany: Dahlem Konferenzen.

Straley, S. C., and V. G. Bruce. 1979. Stickiness to Glass, Circadian changes in the cell surface of *Chlamydomonas reinhardi. Plant Physiol.* **63:** 1175–1181.

Sweeney, B. M. 1974. The potassium content of *Gonyaulax polyedra* and changes in the circadian rhythm of stimulated bioluminescence by short exposure to ethanol and valinomycin. *Plant Physiol.* **53:** 337–342.

Sweeney, B. M. 1976. Evidence that membranes are components of circadian oscillators. Pp. 267–282 in J. W. Hastings and H-G. Schweiger, Eds. *The Molecular Basis of Circadian Rhythms*, Abakon Verlagsgestellschaft, Berlin.

Sweeney, B. M., C. F. Tuffli, and R. H. Rubin. 1967. The circadian rhythm in photosynthesis in *Acetabularia* in the presence of actinomycin D, puromycin and chloramphenicol. *J. Gen. Physiol.* **50:** 647–659.

Taylor, W., V. D. Gooch, and J. W. Hastings. 1979. Period shortening and phase shifting effects of ethanol on the *Gonyaulax* glow rhythm. *J. Comp. Physiol.* **130:** 355–358.

Walz, B., and B. M. Sweeney. 1979. Kinetics of the Cycloheximide-induced phase changes in the biological clock in *Gonyaulax. Proc. Nat. Acad. Sci. U. S. A.* **76:** 6443–6447.

Reference: *Biol. Bull.* **161**: 382–391. (December, 1981)

PRECOCIOUS BREAKDOWN OF THE GERMINAL VESICLE INDUCES PARTHENOGENETIC DEVELOPMENT IN SEA CUCUMBERS*

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ABSTRACT

Meiotic divisions of sea cucumber oocytes can be induced and completed by dithiothreitol (DTT) treatment, but DTT-matured, unfertilized eggs are arrested at the pronucleus stage and do not develop. By pipetting the oocyte suspension, the membrane of the germinal vesicle of the full-grown oocyte ruptured and then disappeared. In immature oocytes the rupture of the germinal vesicle eventually resulted in fragmentation of the oocyte proper without formation of the polar body and the fertilization membrane. In DTT-matured, unfertilized oocytes, artificial rupture of the germinal vesicle before its normal breakdown caused parthenogenetic development following polar body formation. Pipetting after normal germinal vesicle breakdown had no such effects. Pipetting did not cause immediate elevation of the fertilization membrane and pipetted oocytes remained fertilizable. These results indicate that the direct outcome of rupturing the germinal vesicle is not the activation of the oocyte in the usual sense of the term, but a provision of some conditions for the oocyte to initiate cleavage division cycles after the meiotic divisions. It is suggested that the germinal vesicle materials contain some factors that induce the "activation" of the oocyte.

INTRODUCTION

In meiotic maturation the germinal vesicle contents are intermingled with the cytoplasm after disappearance of the nuclear membrane. Several investigators have reported that sperms incorporated into oocytes at the germinal vesicle stage form the male pronucleus only after germinal vesicle breakdown (Hirai, 1976; Moriya and Katagiri, 1976; Usui and Yanagimachi, 1976; Elinson, 1977; Longo, 1978; Schuetz and Longo, 1981). Observations of oocyte-fragments deprived of the germinal vesicle indicate that pronucleus formation and subsequent initiation of cleavage after fertilization require the interaction or mixing of germinal vesicle materials with the cytoplasm after its breakdown (Costello, 1940; Katagiri and Moriya, 1976; Skoblina, 1976).

In sea cucumber oocytes meiotic division can be induced and completed by DTT treatment, independently of fertilization (Maruyama, 1980). However, the DTT-matured, unfertilized eggs are arrested at the pronucleus stage and do not initiate development.

To analyze relationships between the nucleo-cytoplasmic interaction and initiation of cleavage division cycles, I attempted to rupture the germinal vesicle in either immature or DTT-matured unfertilized oocytes and investigated the effects of precocious mixing or interaction of the germinal vesicle contents with the cy-

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Abbreviations: DTT, dithiothreitol; PB, polar body.

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toplasm. Precocious mixing or interaction of the germinal vesicle contents with the cytoplasm in DTT-matured, unfertilized oocytes induced initiation of cleavage division cycles after two cycles of meiotic division. These "activated" eggs developed parthenogenetically to auricularia larvae that appeared morphologically normal.

MATERIALS AND METHODS

Gametes

The sea cucumber *Holothuria leucospilota* was collected near the Seto Marine Biological Laboratory of Kyoto University. Females were isolated from males and cultured in running seawater for 1 day or more. After the body surfaces of the females were washed with fresh water, their gonads were removed, washed twice with artificial seawater (Jamarin U, Jamarin Lab., Osaka), and then minced with fine forceps. Oocytes thus isolated were washed twice with artificial seawater. Oocyte maturation was induced by incubation in 1 m*M* dithiothreitol (DTT) solution for 8–10 min (Maruyama, 1980). Immediately after DTT treatment the oocytes were rinsed two or three times to remove DTT. Oocytes or eggs were cultured in artificial seawater at 27–30°C. In the present study, batches in which 90–99% of oocytes matured by DTT treatment were used. In sperm activation experiments, a sperm suspension was prepared from "dry" sperm prior to insemination.

Rupture of the germinal vesicle (pipetting treatment)

Germinal vesicles of unfertilized oocytes were ruptured by mechanical treatment. About 1 ml of oocyte suspension was aspirated 10–15 times through a pipette with a tip diameter of 700 μm (5 times the oocyte diameter). To avoid wounding the oocyte surface, the minimal amount of pipetting to rupture the germinal vesicle was chosen for each batch of oocytes; and, when damage on the oocyte surface was detected after pipetting, the oocyte suspension was discarded. With this simple method, which will be termed "pipetting", the germinal vesicles of all oocytes, regardless of length of DTT treatment, were disintegrated and then disappeared.

Cinematographic observation

To observe development precisely, the pipetted oocytes, unfertilized or fertilized, were filmed at intervals of 15 sec or 1 min with a Bolex camera equipped with a Nikon CFMA time-lapse instrument. Films were analyzed by a Nac Dynamic Frame Analyzer.

RESULTS

Irreversible changes induced by rupture of the germinal vesicle membrane in immature oocytes (without DTT treatment)

Pipetting ruptured the germinal vesicle membrane in full-grown oocytes (Figs. 1A, 1B), including those with a follicle coat (*cf.* Fig. 2A). Immediate light-microscopic examination revealed that the germinal vesicle usually ruptured in its original position, the center of the oocyte. No damage was found on the oocyte surface. When observed several hours later, the ruptured germinal vesicle was neither reconstructed into its original shape nor became visible. Pipetting did not cause formation of the fertilization membrane and the polar body. About 2 h after pipetting, coaggregation of pinkish pigment granules produced a pink spot near the

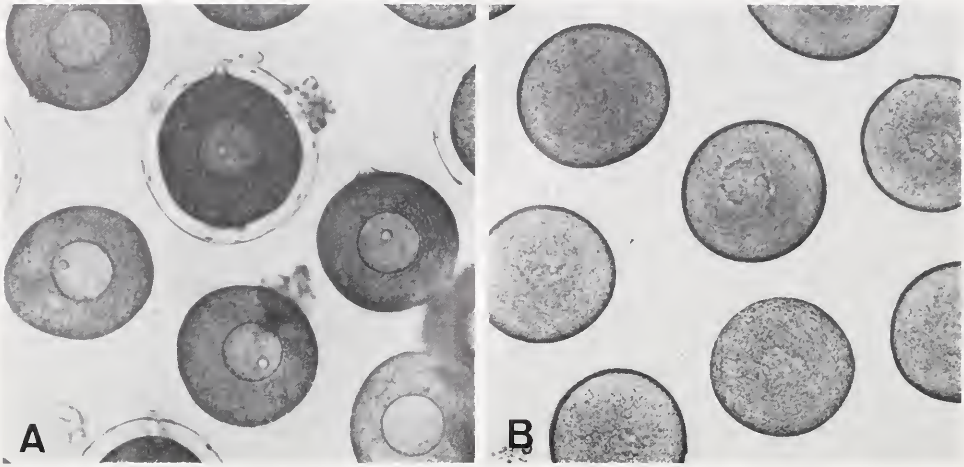

FIGURE 1. Rupture of the germinal vesicle by pipetting. A: intact full-grown oocytes with the germinal vesicle. B: oocytes immediately after pipetting (not induced to mature by DTT).

residue of the germinal vesicle material in some batches of oocytes. Then, all of pipetted oocytes deformed and blebs appeared on the surface. Fragmentation of the oocyte proper followed. By contrast, intact oocytes, with the germinal vesicle, retained their shapes for 20 h or more, even in oocytes that had formed multiple fertilization cones after insemination (Maruyama, 1980).

These results indicate that artificial rupture of the germinal vesicle in immature oocytes induces some irreversible change(s) in physiological activities, leading to fragmentation of the oocyte.

Parthenogenetic development triggered by precocious breakdown of the germinal vesicle in DTT-treated oocytes

Normal events of DTT-induced meiosis in intact, full-grown oocytes are (1) migration of the germinal vesicle to the micropyle process and appearance of a transparent cytoplasmic spot in the subsurface cytoplasm of the antipole of the micropyle process at 15–18 min after the start of DTT treatment at 27–28°C, (2) breakdown of the germinal vesicle at the micropyle process at 20 min, and (3) sequential pinching-off of the first and second polar bodies from the micropyle process (Maruyama, 1980). The unfertilized eggs thus matured were arrested at the pronucleus stage or sometimes at irregular two or four cell stages, and all eggs eventually degenerated through blebbing and fragmentation. In some batches a small percentage (up to 4%) of DTT-matured oocytes developed parthenogenetically.

Artificial rupture of the germinal vesicle by pipetting was also found to be possible at various times after the start of DTT treatment. Pipetting within 20 min after the start of DTT treatment thus produced *precocious* breakdown of the germinal vesicle. The germinal vesicle disintegrated in its original position at the time of pipetting: in the center of the oocyte before 15 min, near the micropyle process between 15 min and 18 min, and at the micropyle process after 18 min. Immediate light-microscopic examination revealed that the oocyte surface was not damaged (see Fig. 2A), although pipetting after 20 min sometimes affected the oocyte surface. The disintegrated germinal vesicle was not reconstructed into its original shape. No elevation of the fertilization membrane was observed, up to 30–50 min after the start of DTT treatment.

A proportion of DTT-treated, unfertilized oocytes whose germinal vesicles had been broken precociously were found to develop parthenogenetically (Fig. 2). Figure 3 shows relationships between the time of pipetting and the frequency of blastulae or gastrulae that were induced to develop parthenogenetically. The efficiency of pipetting in triggering this development was time-dependent. Pipetting at 10 min after the start of DTT treatment gave the maximum efficiency; 20–77% of the pipetted oocytes developed parthenogenetically. Pipetting of intact oocytes at 20 min before DTT treatment was also effective in some batches of oocytes (Fig. 3). However, pipetting at the stages after the normal germinal vesicle breakdown had no effect in triggering the development; these eggs became fragmentated after two cycles of meiotic division.

In the following experiments, pipetting was made routinely at about 10–11 min after the start of DTT treatment.

When maturation of oocytes pipetted at 10–11 min following DTT treatment was observed with time-lapse cinematography, the residue of the germinal vesicle material could be distinguished from the surrounding cytoplasm as a clear area. At 15–20 min after the start of DTT treatment (corresponding to the stage of germinal vesicle migration in control oocytes), the residue of the ruptured germinal vesicle in the pipetted oocytes also migrated to the micropyle process. Almost coincidently, a transparent cytoplasmic spot (termed "clear spot") was formed in

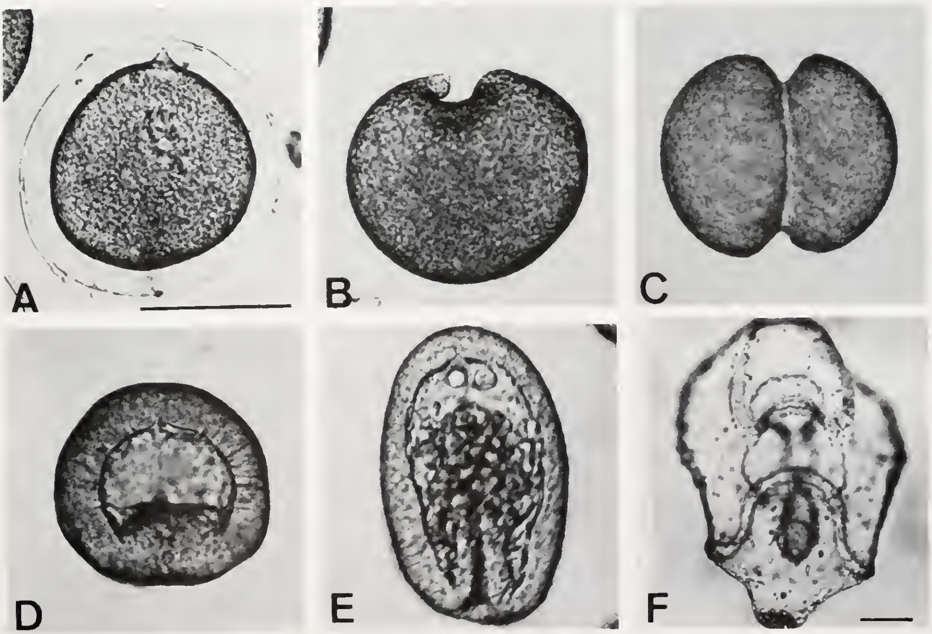

FIGURE 2. Parthenogenetic development of pipetted, DTT-treated oocytes. Oocytes were treated with DTT and then pipetted at 10–11 min after the start of DTT treatment. A: oocyte immediately after pipetting. The follicle coat was stripped off later. B: unilateral deformation (DEF) in an egg forming both polar bodies. C: first cleavage. D: gastrula at 16 h. E: late gastrula with mesenchyme cells at 24 h. F: auricularia at 4.5 days. Bars: 100 μm (the same magnification from A to E).

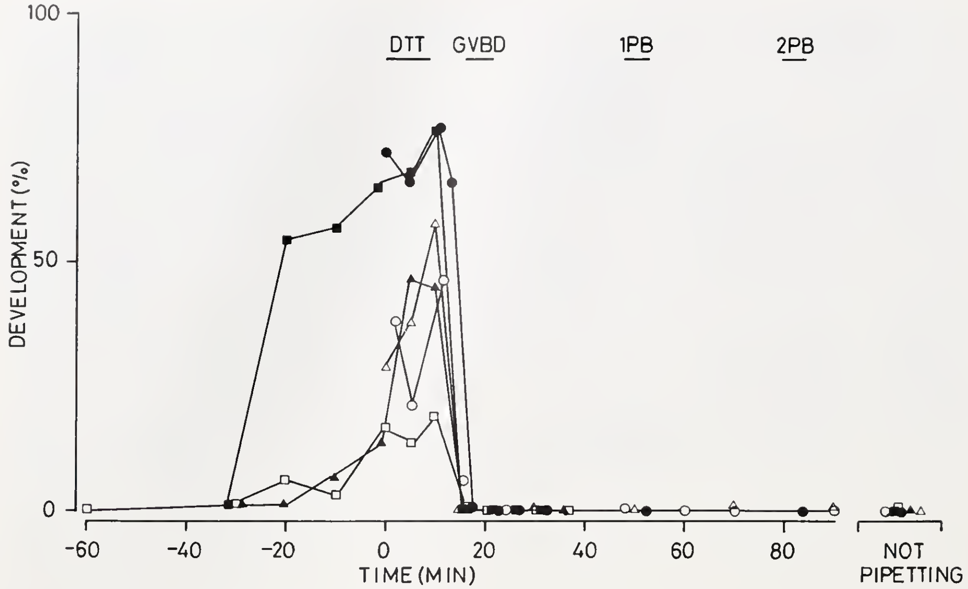

FIGURE 3. Frequencies of parthenogenetic development in unfertilized oocytes pipetted at various times before, during, or after DTT treatment. Abscissa: time (min) of pipetting before or after the start of DTT treatment. Not-pipetting shows controls that were matured by DTT, but not pipetted. Ordinate: percentage of pipetted oocytes developing to blastulae or gastrulae by 11–18 h; DTT, duration of DTT treatment (about 9 min); GVBD, germinal vesicle breakdown in not-pipetted controls; 1PB, first polar body; 2PB, second polar body. Results in 6 batches of oocytes are shown. Each point represents about 100 oocytes. Temperature was 29–30°C.

the subsurface cytoplasm of the antipole of the micropyle process. Then, from 30 min to 50 min, a considerable proportion of the pipetted, unfertilized, and DTT-treated oocytes were observed to elevate the fertilization membrane "spontaneously." This elevation of the fertilization membrane followed transient surface wrinkling, just as in inseminated, DTT-matured controls. The fertilization membrane subsequently sank and adhered to the egg surface.

Table I shows polar body formation in pipetted, unfertilized, and DTT-treated oocytes. In 68% of the oocytes, the first and second polar bodies were formed from the micropyle process, nearly at the same time as in DTT-matured controls. In 24% of the oocytes, no formation of the second polar body was observed. In 8% of the oocytes, both polar bodies were not formed. In this way, most of the oocytes (92%) formed polar bodies. Most oocytes forming one or two polar bodies began parthenogenesis as shown in Table I and Figure 4. These results indicate that failure of polar body formation itself does not causally relate to initiation of cleavage division cycles in these pipetted oocytes.

Figure 4A shows timetables of parthenogenesis in the pipetted, unfertilized, and DTT-treated oocytes (shown in Table I) that formed two polar bodies (2PB), one polar body (1PB), or no polar bodies (0PB).

2PB eggs. An event specific to the eggs was transient formation of a unilateral deformation (DEF) (Fig. 2B), with accumulation of pinkish pigment granules in the deformed region. This appeared at about 130 min and regressed at 150 min when the eggs resumed a spherical shape with dispersal of the accumulated pigments. The deformed region appeared random as to the position of polar bodies

TABLE I

Polar body formation in pipetted, unfertilized, and DTT-treated oocytes

	No. of oocytes observed		
Batch	2PB	1PB	0PB
A	15 (10)	5 (0)	0 (0)
B	9 (6)	7 (4)	5 (5)
C	19 (15)	3 (3)	0 (0)
Totals	43 (31)	15 (7)	5 (5)
% of totals	68 (72)	24 (16)	8 (12)

2PB, 1PB, and 0PB, respectively, indicate oocytes forming the first and second polar bodies, those forming one polar body, and those forming no polar body. These oocytes were continously observed from 20 min to 210 min after the start of DTT treatment at 29–30°C. Numerals in parentheses show the number of oocytes which began parthenogenesis in the population of oocytes observed. Pipetting was made at 10–11 min.

on the oocyte surface. About 30–35 min after appearance of the deformation, first cleavage (Fig. 2C) occurred as in inseminated control eggs. At that time, a similar deformation appeared again in a small percentage (about 6%) of these eggs. Subsequent cleavages took place at intervals of 30–35 min.

1PB eggs. Eggs failed to form the second polar body, and surface deformation near the micropyle process was observed nearly at the same time as second polar body formation in the controls. At about 135 min, first cleavage took place as in the inseminated control eggs. Subsequent cleavages occurred at intervals of 30–35

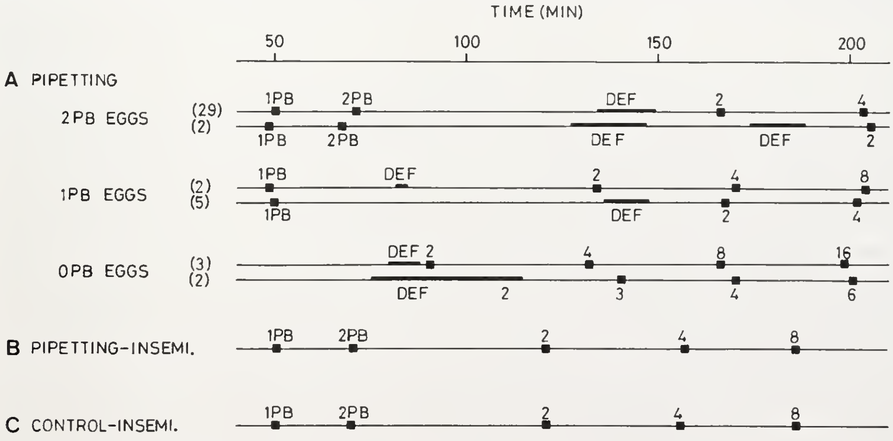

FIGURE 4. Timetables of developmental events in pipetted, unfertilized oocytes (A); pipetted, inseminated oocytes (B); control, inseminated oocytes (C). In each event the mean of the initiation time was indicated by the closed box. All oocytes were treated by DTT for 9–10 min. Pipetting was at 10–11 min for A and B, and insemination was at 23 min for B and C. Abscissa: time (min) after the start of DTT treatment. 1PB, first polar body; 2PB, second polar body; DEF, unilateral deformation; Arabic number, stages expressed by cell number. Numerals in parentheses in A indicate the number of oocytes observed; these were the identical oocytes as shown in parentheses in Table I. Temperature was 29–30°C.

min. In most of the eggs (5 out of 7), such a unilateral deformation as observed in 2PB eggs took place at 135–150 min, and first cleavages in these occurred 30–35 min later.

0PB eggs. The first and second polar bodies were not formed. Immediately after second polar body formation in the inseminated control eggs, a unilateral furrow-like constriction appeared, and then the eggs divided into two cells with one nucleus in each cell. Most of them became four cells at about 130 min. In some only one of the two cells divided producing a three cell stage. Subsequent cleavages occurred at intervals of 30–35 min. This group of eggs was considered at the most advanced "stages" in the parthenogenetically developing egg population.

As shown in Figure 4, timing of each cleavage or deformation was similar, except for the two cell stage in 0PB eggs, which was delayed 10–20 min as compared to control inseminated eggs. Their intervals between cleavages (deformation) were similar to those in control inseminated eggs. Most of these cleaving eggs developed to gastrulae. The gastrulae began to move by ciliary motion and hatched from the fertilization membrane.

Further development in the pipetted, unfertilized, and DTT-treated eggs was similar to that in normally fertilized eggs. Figures 2D and 2E, respectively, show free swimming early and late gastrulae. They swam with the animal pole leading, accompanied with counter-clockwise spin as in normally developing embryos (Maruyama, 1980). Later, the dorsal pore and pore canal formed; the dorsal pore shifted to the left side from the middle line of the body, seen from the dorsal side of the body. By 3 days, they developed to typical auricularia larvae (Fig. 2F). A birefringent body formed at the posterior end of the body and the ciliary ridge clearly formed a single loop.

Fertilizability and post-fertilization development in DTT-treated, pipetted oocytes

DTT-treated oocytes with germinal vesicles ruptured precociously by pipetting at 10–11 min were inseminated at various times after the start of DTT treatment. Insemination from 23 min to 40 min caused immediate surface wrinkling, formation of the fertilization cone, and elevation of the fertilization membrane in all of the oocytes. Most of these oocytes (70–100%) had a single fertilization cone. However, insemination after 50 min did not cause surface wrinkling and formation of the fertilization cone. Most of the pipetted, uninseminated, and DTT-treated oocytes elevated fertilization membranes at 50 min. By contrast, at these times, not-pipetted, DTT-matured oocytes were fertilized as evidenced by surface wrinkling, fertilization cone, and elevation of the fertilization membrane upon insemination. In this way, the pipetted, DTT-treated oocytes were fertilized when inseminated before their "spontaneous" elevation of the fertilization membrane.

Figure 5 shows timing of first cleavage in DTT-treated oocytes that were pipetted at 10–11 min and inseminated at 23 min after the start of DTT treatment. First cleavages occurred at the same time as in the control DTT-matured oocytes that had been inseminated at 23 min. The subsequent cleavages took place at the same time as in control inseminated oocytes (Figs. 4B, 4C). These pipetted, inseminated oocytes showed no pre-cleavage deformation. Most of them (80%) developed to gastrulae by 14 h. This contrasts with the pipetted, uninseminated oocytes, in which only 50% (Fig. 5) developed with some delay, after pre-cleavage deformation.

These results indicate that DTT-treated, pipetted oocytes can be fertilized by

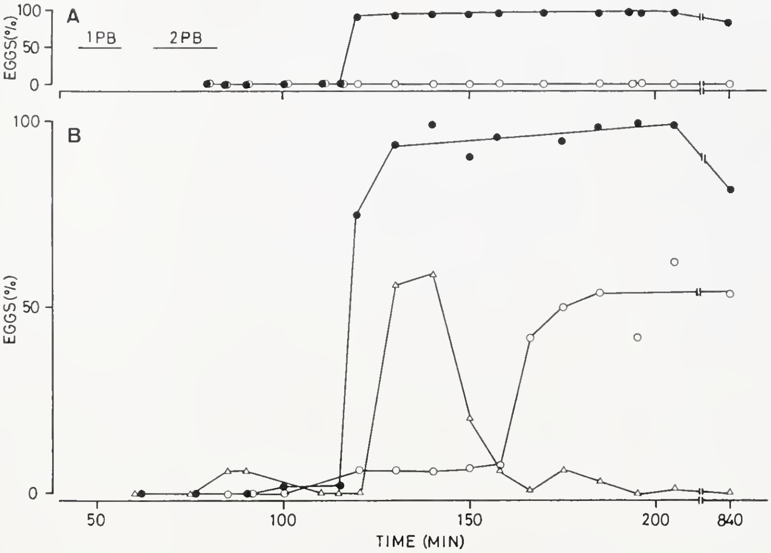

FIGURE 5. Effects of sperm activation in DTT-treated, pipetted oocytes and control oocytes. Abscissa: time (min) after the start of DTT treatment. Ordinate: percentage of cleaving eggs. A: control oocytes which were inseminated at 23 min (closed circles), or not inseminated (open circles), following DTT treatment for 9 min. B: oocytes that were pipetted at 10–11 min following DTT treatment and were either inseminated at 23 min (closed circles) or not inseminated (open circles). The open triangles show the oocytes that exhibit unilateral deformation among the pipetted, unfertilized oocytes. The oocytes derived from the same batch were used for A and B. Each point represents about 100 oocytes.

sperm, and that fertilization switches the pattern of development to that in normally fertilized oocytes.

DISCUSSION

The present study shows that (1) the germinal vesicle in sea cucumber oocytes can be ruptured by pipetting, (2) oocytes with ruptured germinal vesicles change their physiological activities irreversibly, (3) the pipetted, unfertilized oocytes can be induced to mature by DTT treatment, and, (4) when the germinal vesicle is ruptured before its normal breakdown, most of the DTT-treated oocytes begin parthenogenetic development after meiosis.

This pipetting method differs in an essential feature from previously reported methods of inducing parthenogenesis in echinoderm eggs (Lillie, 1915; Harvey, 1956; Epel, 1979). In these methods the effective period of activation treatment coincides with a period during which eggs can be triggered to develop by normal fertilization. Responses of the eggs following the artificial activation, therefore, are similar, in their properties and timing, to those in eggs activated by fertilization. In the pipetting method of parthenogenesis, the effective time for the treatment was before the normal fertilizable time, or before breakdown of the germinal vesicle (see Maruyama, 1980).

The primary target of the pipetting treatment may be the egg surface. However, due to the thick jelly coat and follicle coat surrounding the oocyte, aspiration of the oocytes through the pipette did not seem to affect the integrity of the oocyte surface. The only change observed was rupture of the germinal vesicle membrane.

Capability of monospermic fertilization, as evidenced by the single fertilization cone and elevation of the fertilization membrane in DTT-treated, pipetted oocytes upon insemination, may also indicate that pipetting itself does not directly cause a cortical reaction. On the other hand, the time-dependence of efficiency of pipetting for inducing parthenogenesis indicates that a meiotic phase is important in triggering parthenogenetic development. Precocious "breakdown" of the germinal vesicle may be the principal cause of parthenogenetic development. It may be concluded that its resultant precocious interaction (or mixing) of the germinal vesicle contents with the cytoplasm causes initiation of cleavage division cycles.

DTT-treated, unfertilized oocytes were arrested at the pronucleus stage or sometimes at irregular two or four cell stages. Naturally spawned oocytes were also arrested at the pronucleus stage after two cycles of meiotic division when they were not inseminated, and all of them eventually fragmentated (Maruyama, unpublished). These observations may suggest presence of some mechanisms that inhibit initiation of cleavage division cycles after meiosis and can be normally triggered by fertilization, as the mechanism of metaphase II arrest in amphibian oocytes (Masui and Clarke, 1979). Unfertilized oocytes whose germinal vesicles had been broken either by DTT maturation in oocytes not-pipetted, by natural maturation, or by pipetting in immature oocytes were not arrested at some stable states but became fragmentated eventually. These observations and "spontaneous" elevation of the fertilization membrane accompanying surface wrinkling in pipetted, unfertilized, and DTT-treated oocytes suggest that the germinal vesicle materials contain some factors that activate physiological occurrences in the oocyte. When this activation occurs at a suitable time during the maturation process induced by DTT treatment, the oocytes may begin parthenogenetic development. As shown by fertilizability of pipetted, DTT-treated oocytes, however, this "activation" does not directly activate the oocyte in the usual sense of the term, but is a provision of some condition for the oocytes to initiate parthenogenesis.

First cleavage in pipetting-induced parthenogenesis occurred considerably later than in normally fertilized eggs and its timing varied among types of eggs as to the number of polar bodies formed (Fig. 4). However, cleavages or deformation occurred at the same time among 2PB, 1PB, and 0PB eggs (Fig. 4). Such coincidence of their timing suggests that these eggs were determined to initiate parthenogenetic development at an identical point in time.

Time-dependence of pipetting to induce parthenogenesis in DTT-treated oocytes, and irreversible changes of physiological activities in immature oocytes with ruptured germinal vesicles indicate that untimely disappearance of the nuclear membrane during cell cycle may affect normal expression of cytoplasmic activities. Thus, the nuclear membrane probably functions as a barrier against untimely exposure of the karyoplasm (or chromosomes) to its cytoplasm.

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LITERATURE CITED

Costello, D. P. 1940. The fertilizability of nucleated and non-nucleated fragments of centrifuged *Nereis* eggs. *J. Morph.* **66:** 99–114.

Elinson, R. P. 1977. Fertilization of immature frog eggs: cleavage and development following subsequent activation. *J. Embryol. Exp. Morph.* **37**: 187–201.

Epel, D. 1979. The triggering of development at fertilization. Pp. 17–31 in J. D. Ebert and T. S. Okada, Eds. *Mechanisms of cell change.* John Wiley and Sons, New York. 343 pp.

Harvey, E. B. 1956. *The American* Arbacia *and other sea urchins.* Princeton University Press, New Jersey. 298 pp.

Hirai, S. 1976. The role of germinal vesicle material on the swelling of sperm nuclei penetrated in immature oocytes of the starfish. *Bull. Marine Biol. St. Asamushi* **15**: 165–171.

Katagiri, Ch., and M. Moriya. 1976. Spermatozoan response to the toad eggs matured after removal of germinal vesicle. *Dev. Biol.* **50**: 235–241.

Lillie, R. S. 1915. Momentary elevation of temperature as a means of producing artificial parthenogenesis in starfish eggs and the conditions of its action. *J. Exp. Zool.* **5**: 375–428.

Longo, F. J. 1978. Insemination of immature sea urchin (*Arbacia punctulata*) eggs. *Dev. Biol.* **62**: 271–291.

Maruyama, Y. K. 1980. Artificial induction of oocyte maturation and development in the sea cucumbers *Holothuria leucospilota* and *Holothuria pardalis. Biol. Bull.* **158**: 339–348.

Masui, Y., and H. J. Clarke. 1979. Oocyte maturation. *Int. Rev. Cytol.* **57**: 185–282.

Moriya, M., and Ch. Katagiri. 1976. Microinjection of toad sperm into oocytes undergoing maturation division. *Dev. Growth and Differ.* **18**: 349–356.

Schuetz, A. W., and F. J. Longo. 1981. Hormone-cytoplasmic interactions controlling sperm nuclear decondensation and male pronuclear development in starfish oocytes. *J. Exp. Zool.* **215**: 107–111.

Skoblina, M. N. 1976. Role of karyoplasm in the emergence of capacity of egg cytoplasm to induce DNA synthesis in transplanted sperm nuclei. *J. Embryol. Exp. Morph.* **36**: 67–72.

Usui, N., and R. Yanagimachi. 1976. Behavior of hamster sperm nuclei incorporated into eggs at various stages of maturation, fertilization, and early development. *J. Ultrastruct. Res.* **57**: 276–288.

Reference: *Biol. Bull.* **161:** 392–401. (December, 1981)

SPECIFIC DISINTEGRATION OF NEMATOCYSTS IN *HYDRA JAPONICA* BY TREATMENT WITH PROPARGYLGLYCINE

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ABSTRACT

When *Hydra japonica* was treated with 2 m*M* D,L-c-propargylglycine, a specific inhibitor of γ-cystathionase, for 75 h, the number of desmonemes present in the tentacles dropped drastically. H. isorhiza and A. isorhiza numbers also showed smaller drops, but the stenotele number remained unchanged. Total cell numbers and cell composition except for the battery cells and nematocytes, remained unchanged. The disappearance of the mature desmonemes was apparently due to their specific disintegration in the living hydra, and not to inhibition of differentiation from interstitial cells to nematocytes, and/or their migration, nor accelerated nematocyst discharge from the battery cells. Hydra lacking desmonemes may be useful in investigating how the proportions of each nematocyte type are kept constant, and how nematocyte differentiation is regulated.

INTRODUCTION

Hydras have many nematocytes, which are classified into four types: stenotele, desmoneme, atrichous isorhiza (A. isorhiza), and holotricous isorhiza (H. isorhiza), according to their structure and functions (Mariscal, 1974). They differentiate from the interstitial cells in the intercellular space of the body ectoderm (David and Challoner, 1974). Most mature nematocytes, especially desmonemes and A. isorhizas, migrate to the tentacles and are retained in the battery cells (Campbell, 1967; David and Gierer, 1974). The ratio of nematocytes to interstitial cells and the proportions of each nematocyte type are kept constant throughout asexual growth (Bode *et al.*, 1977). The mechanism for keeping the stem-cell pool constant has been investigated (Bode and David, 1978), but the mechanism which keeps the proportions of each nematocyte type constant is unknown. Hydras that lack a particular type of nematocyte may be useful in investigating this latter mechanism.

In studying nematocyte differentiation, we found that the number of nematocyst capsules in the tentacles decreased drastically when hydras were treated with D,L-c-propargylglycine (PG), an inhibitor of γ-cystathionase, the enzyme for L-cysteine biosynthesis (Washien and Abeles, 1977). The effects of PG on hydras, especially on nematocysts, were then examined in detail. We found that desmonemes disappeared because they disintegrated. This treatment provides a method of preparing large numbers of hydras lacking desmonemes and A. isorhizas.

MATERIALS AND METHODS

Culture of hydra and D,L-c-propargylglycine treatment

The *Hydra japonica* used for all experiments were mass cultured in a solution containing 1.0 m*M* $NaHCO_3$, 0.3 m*M* $CaCl_2$, 0.06 m*M* KCl, 0.06 m*M* $MgCl_2$,

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Abbreviations: PG: D,L-c-propargylglycine; SDS: Sodium dodecyl sulphate; H. isorhiza: Holotrichous isorhiza; A. isorhiza: Atrichous isorhiza.

and 0.1 m*M* EDTA·4Na (pH 7.8–8.0) in distilled and deionized water at 20(±1)°C according to the methods of Hirakawa and Kijima (1980). Large hydras without buds, which had been starved for 2 days, were exposed to 2 m*M* D,L-c-propargylglycine (PG) in the culture medium for various periods. The medium was changed daily and animals were not fed during the experimental period.

Counting of nematocysts and cell type

Twenty hydras were transferred to a tube and rinsed with about 5 ml culture medium. A nematocyst suspension was prepared by dissolving the hydras in 0.5 ml or 1.0 ml culture medium containing 2% sodium dodecyl sulphate (SDS). Total nematocysts and the number of each kind in the suspension, then, were counted with a blood-corpuscle counting chamber under a phase contrast microscope at ×400 (Bode and Flick, 1976). The error bars in the figures represent the standard errors of means in three to five experiments.

Hydra was macerated to quantify each cell type. Maceration and cell identification followed the procedure of David (1973).

RESULTS

Effects of PG on morphology and behavior

The tentacles of animals exposed to PG became shorter and smoother than untreated tentacles. Figure 1 shows part of a hydra tentacle treated with 2 m*M* PG for 72 h and a control tentacle. Battery cells in the tentacles of a control hydra contained many nematocytes with one cnidocil on the apex of each nematocyte. One stenotele at the center of the battery cell was surrounded by about 15 desmonemes. A few H. isorhizas and A. isorhizas also were often in the battery cell (Fig. 1a). In the tentacles of treated hydras (Fig. 1b), most of the desmonemes and their cnidocils disappeared, but stenoteles remained unchanged. The nematocyst capsules remaining in the tentacles seemed a little smaller, and the thread forming a corkscrew like coil in the desmoneme's capsule was also not visible.

The behavior of treated hydra (such as spontaneous column and tentacle contractions) was almost normal except for the feeding behavior and foot attachment. The feeding behavior, which is induced by prey or with glutathione, almost completely stopped. Normally, after feeding hydras adhere to the bottom, while starved hydras make a bubble at the foot, and float on the surface of the culture medium under the culture conditions employed. The PG-treated hydra, however, became adhesive to the bottom.

Effect of PG on number of nematocysts

A single untreated *H. japonica* had about 32,000 nematocytes, of which 76% were desmonemes, 10% stenoteles, 7.5% H. isorhizas, and 6.5% A. isorhizas. Each type of nematocyst was counted at various times during the treatment of hydra with 2 m*M* PG (Table I and Fig. 2). The number of each type barely decreased within the first 25 h of PG-treatment. Subsequently, the number of desmonemes decreased sharply to 20% of the control value at 75 h after the application of 2 m*M* PG (Fig. 2). A. isorhizas also decreased a little more slowly than desmonemes. H. isorhiza number decreased still more slowly, to 52% of control number in 50 h. On the other hand, the stenoteles did not decrease significantly during the 75

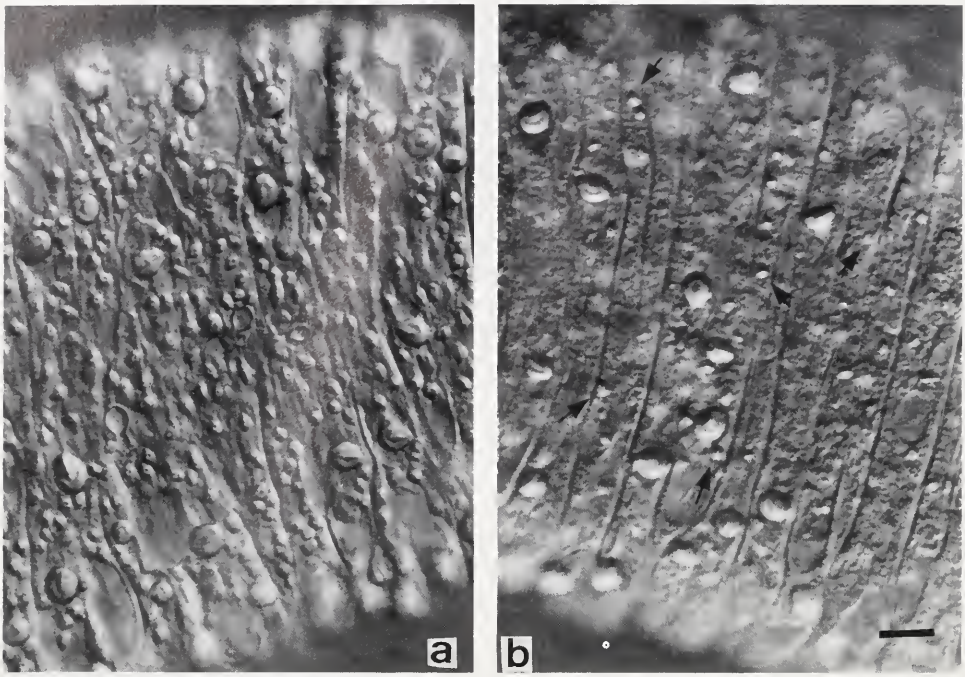

FIGURE 1. Photomicrographs of hydra tentacle observed under Normarski differential interference optics. a) control. b) 2 m*M* treatment for 75 h. Arrows indicate the few remaining and deformed desmonemes after treatment. Scale bar equals 20 μm.

h treatment, but after the 150 h treatment stenoteles decreased to 43% of control level (Table I).

About 80% of all nematocysts were in the tentacles. This included 90% of desmonemes and 80% of A. isorhizas. About 70% of the stenoteles and 55% of the H. isorhizas, in contrast, were in the body. To examine whether the decrease in nematocyst numbers occured mainly in the tentacles, hydras were cut into two pieces just below the hypostome region, and the numbers of nematocysts in each portion counted. In the tentacles, the numbers of desmonemes, A. isorhizas, and H. isorhizas dropped to about 10%, 25% and 30% of control level, respectively, after treatment with 2 m*M* PG for 75 h. The number of stenoteles did not decrease. In the body, the numbers of each kind of nematocyst were almost the same as in the controls. This showed that, except for the stenoteles, PG treatment decreased the numbers of nematocysts in the tentacles, but did not affect nematocysts in the body during the 75 h treatment.

Effect of PG on the cell population

The total cell number, excluding the nematocytes, in a typical hydra was 7 $\times 10^4$: about 40% epithelial cells, 37% interstitial cells, 7% nerve cells, and 16% other cell types (gland cells, mucous cells and nematoblast cells). PG did not change the total cell number except for nematocytes in the battery cells during 100 h

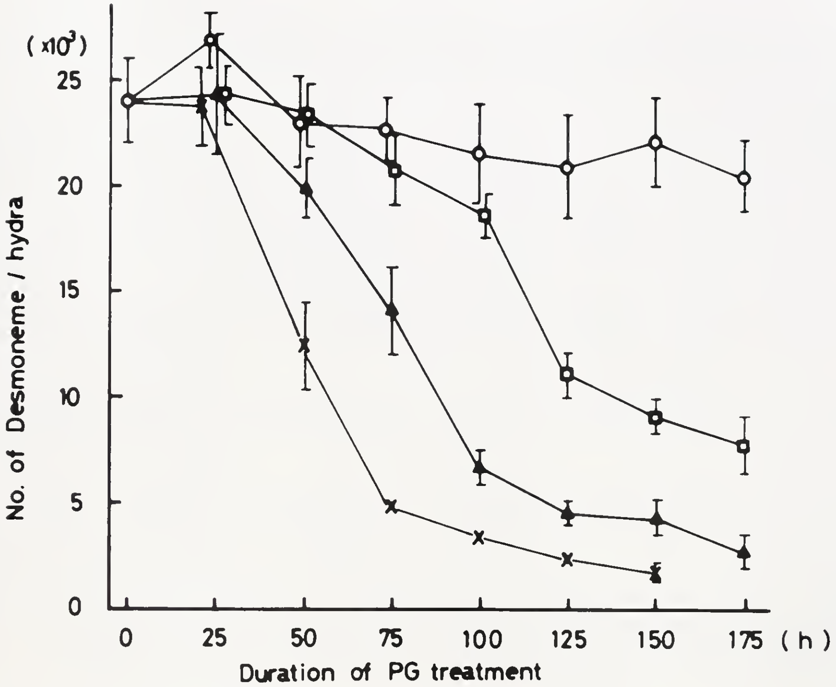

FIGURE 2. Time course of PG effects on desmonemes in a whole hydra. (○, control; □, 0.5 m*M*; △, 1.0 m*M*; ×, 2.0 m*M*)

treatment. The proportions of each cell type were also little different from those in the untreated hydras.

Effect of PG on hydras in which the supply of desmonemes was blocked by cutting

In normal hydra, the number of nematocytes is kept nearly constant by balanced production and depletion (Bode and Flick, 1976). The decrease of the desmonemes in PG-treated hydra could be caused by inhibition of production, or acceleration of depletion, or both. Nematoblast cells differentiating to desmonemes are located in the gastric region, and absent just under the hypostome and in the tentacles (David and Challoner, 1974). Thus, the supply of newly matured desmonemes to the tentacles can be stopped for at least 60 h by removing the hydra's body just under the hypostome. The interstitial cells remaining in the hypostome might start to differentiate into desmonemes just after cutting, but it should take more than 60 h for differentiation from the final cell division to the matured nematocyte (David and Gierer, 1974).

Hydras were divided into two groups, I and II. Group I consisted of intact hydra while group II consisted of tentacles and hypostome only. Half of the animals of each group were incubated in PG (these were designated as PG-I and PG-II). The tentacles of group II animals (Control-II and PG-II) were not supplied with new desmonemes, while those of group I (Control-I and PG-I) were supplied during

TABLE I

*The effect of 2 m*M *PG on the numbers of nematocysts in a hydra.*

Duration of PG treatment		Desmoneme	Stenotele	Holo-trichous Isorhiza	Atrichous Isorhiza	Total Nematocyst
0 h	C	24,000 ± 2,000	3,050 ± 300	2,400 ± 450	2,000 ± 200	31,500 ± 2,650
25 h	C	26,900 ± 1,200	3,900 ± 450	2,450 ± 300	2,200 ± 400	35,500 ± 3,600
	PG	23,800 ± 1,800	3,150 ± 200	2,450 ± 400	1,800 ± 250	31,200 ± 2,300
	%	88.5	80.8	100	81.3	88.0
50 h	C	24,700 ± 2,100	3,800 ± 450	2,400 ± 300	2,000 ± 350	32,900 ± 3,000
	PG	12,450 ± 2,050	4,450 ± 500	2,200 ± 400	1,050 ± 250	20,150 ± 2,400
	%	50.5*	118.7	91.7	52.5*	61.2*
75 h	C	28,800 ± 4,600	3,900 ± 600	2,500 ± 400	2,050 ± 300	37,400 ± 5,750
	PG	4,850 ± 950	3,800 ± 400	1,300 ± 100	600 ± 150	10,600 ± 750
	%	16.8*	97.4	52.0	29.3*	28.3*
150 h	C	23,700 ± 2,100	4,650 ± 550	2,500 ± 400	1,700 ± 200	32,600 ± 2,900
	PG	1,700 ± 900	2,000 ± 200	500 ± 100	350 ± 100	4,550 ± 550
	%	7.2*	43.0*	20.0*	20.6*	14.0*

Data are the cumulative results of 5 or 6 independent experiments and are presented as mean ± SEM.

* Significantly different from untreated hydra at the 95% confidence level using *t*-test.

Abbreviations: C: untreated with PG, PG: treated with PG, %: per cent of PG to C.

the experimental period. If PG only inhibits the production of desmonemes and/or their migration to the tentacles, the number of desmonemes of PG-II should be the same as that of Control-II. On the other hand, if the decrease is due to depletion from the tentacles only, the rate of decrease in PG-II should be the same as that in PG-I. The numbers of desmonemes in the tentacles and hypostome in each of the four groups were counted after the intervals shown in Figure 3. For 30 h of treatment, from 20 h to 50 h, Control-I and Control-II remained almost unchanged. The numbers of desmonemes in PG-I and PG-II, however, showed a similar and drastic decrease. Thus, the rapid decrease of desmonemes was mainly due to acceleration of depletion rather than the inhibition of production or migration.

Effect of PG on the discharge of desmonemes

Two possible mechanisms for the depletion of desmonemes in the tentacles are (1) discharge of desmonemes into the external medium, and (2) their disintegration in the living tentacles. The mature nematocyst-capsule was so stable in the culture medium that all types of nematocysts remained intact for at least 3 days. Therefore, the discarded nematocysts could be estimated by counting nematocysts in the culture vessels. However, direct counting of discharged nematocysts was difficult, since their number was very small and nematocyst threads got tangled together when discharged nematocysts were enriched by centrifuging. So, the total number of nematocysts (both discarded and retained in the hydra) was counted and compared with the number of retained nematocysts only at various times during PG treatment. Figure 4 shows that the sum of the discarded and retained desmonemes was almost the same as the number of retained desmonemes in both control and PG treated

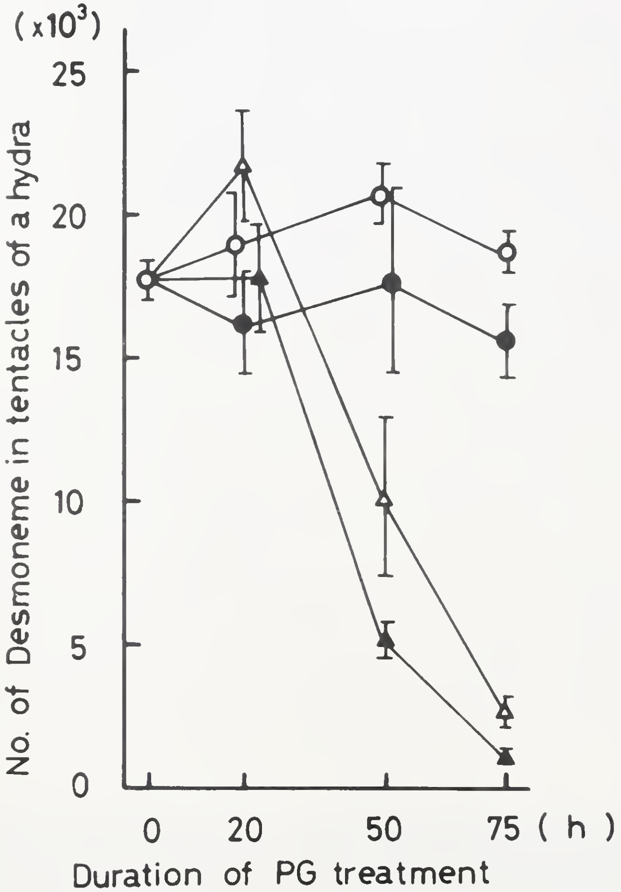

FIGURE 3. Effect of 2 mM PG on the desmonemes of hydra tentacles and hypostome with blocked supply of new desmonemes. Hydras in Control-I (○) and PG-I (△) were cut just under the hypostome immediately before counting, while animals in Control-II (●) and PG-II (▲) were cut just before the PG-treatment (at time 0), to block the supply of new nematocysts. Control-I and -II were not treated with PG.

hydras. This shows that the number of discarded desmonemes is negligibly small. The sum of retained and discarded desmonemes in treated hydras decreased markedly compared to controls. Thus, the decrease in desmonemes was not due to their discharge, but due to disintegration in living hydras.

Hydra with a few drops of 2 mM PG-solution were placed on a glass slide, and nematocysts were discharged by touching the hydra with a glass rod. The discharged nematocysts were observed under the microscope for 3 days. The nematocysts remained unchanged and did not disintegrate by direct action of PG. Thus, PG may act on a mechanism which maintains mature desmonemes in the living hydras, and the desmonemes may be degraded by metabolic action of living cells. This is supported by the fact that many small objects thought to be degrading capsules were observed in the living tentacles of PG-treated hydra (Fig. 1b) and in the solution where PG-treated hydras were dissolved by 2% SDS (Fig. 5b).

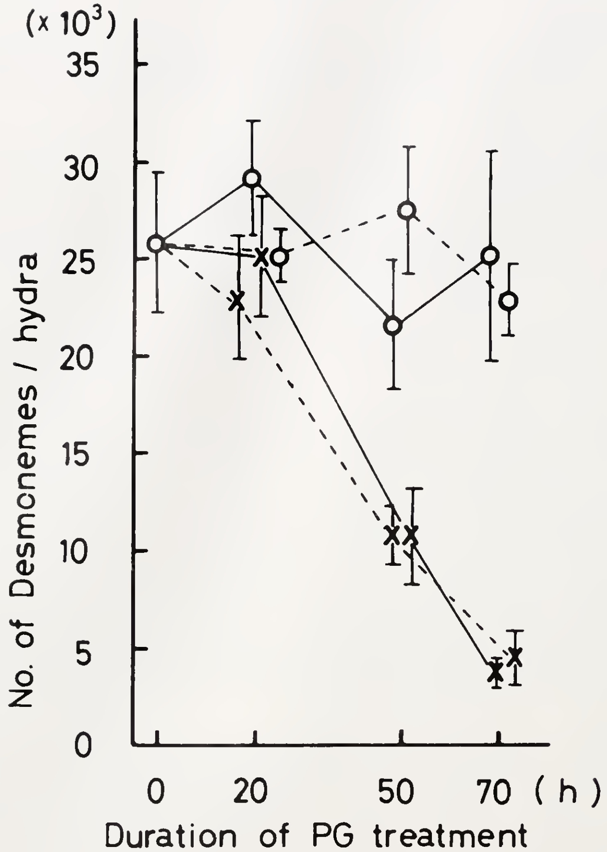

FIGURE 4. Effect of 2 m*M* PG on the discharge of desmonemes. Hydras were treated with 2 m*M* PG. At 0 h, 20 h, and 50 h of the treatment, twenty hydras were transferred to a separate tube, rinsed with about 5 ml test medium and were cultured in 0.4 ml test medium for 20, 30 or 20 hrs, respectively. 0.1 ml of 10% SDS was then added and the numbers of desmonemes counted (times 20 h, 50 h, and 70 h, respectively). These values represented the sum of both discarded and retained desmonemes (- - O - -, - - × - -). The number of retained desmonemes only (— O —, — × —) was counted at 0 h, 20 h, 50 h, and 70 h by transferring twenty hydra to a separate tube, rinsing and immediately adding 10% SDS. Open circles represent numbers of desmonemes in control animals and crosses represent those treated with 2 m*M*.

Reversibility of PG effect

When hydras were transferred back to normal culture medium after 3 days of treatment, the decrease of nematocysts stopped. If transferred hydras were force-fed, increased nematocyte numbers and budding occurred. Nematocyst numbers returned to control levels in a week indicating that budding and nematocyte synthesis ability were not destroyed permanently by the PG treatment. The details of the recovery process will be reported elsewhere.

DISCUSSION

The experiments presented show that PG treatment drastically decreased the number of nematocysts, especially desmonemes, mainly due to the disintegration of the nematocysts in living tentacles. PG's action differed depending on the type of nematocysts and their location. Stenoteles showed the least response to PG. All nematocyst types in the body column were not affected. Some nematocysts, including most desmonemes and A. isorhizas, migrate and do not remain in the body column. These results suggest that PG acts after the nematocyte migration.

The following results were obtained concerning the mechanism of nematocyte disintegration. (1) The capsules of remaining nematocysts became slightly smaller and were deformed (Fig. 1). The deformed nematocysts were often found in SDS suspension (Fig. 5). (2) The cnidocils of nematocytes on the tentacle disappeared after PG treatment. Sections of treated hydra showed few nematocyte nuclei under phase contrast microscopy. Thus, the capsule and the nematocyte may disintegrate almost simultaneously after a little shrinkage of the nematocyst capsule.

Campbell (1976) reported that colchicine treatment depleted the interstitial cells, nematoblasts, nematocytes, and nerve cells in *H. attenuata*, and that this depletion was due to phagocytosis by the digestive cells in the body. However, such phagocytosis probably is not responsible for the PG depletion of desmonemes because PG had little effect on desmonemes in the body region. Desmonemes were degraded in the tentacle where few digestive cells are located.

Mature nematocytes are discarded into the external solution after nematocyst discharge, or otherwise, nematocytes migrate to the tentacle tip and are discarded from the tip. Presumably, they never disintegrate inside normal living hydra. Even in a mutant hydra which synthesized stenoteles that did not migrate into the tentacles (Fujisawa and Sugiyama, 1978), stenoteles which accumulated in the body, presumably, did not disintegrate but were discarded into the gastroderm. However, PG treatment caused disintegration of mature nematocyst in living hydra.

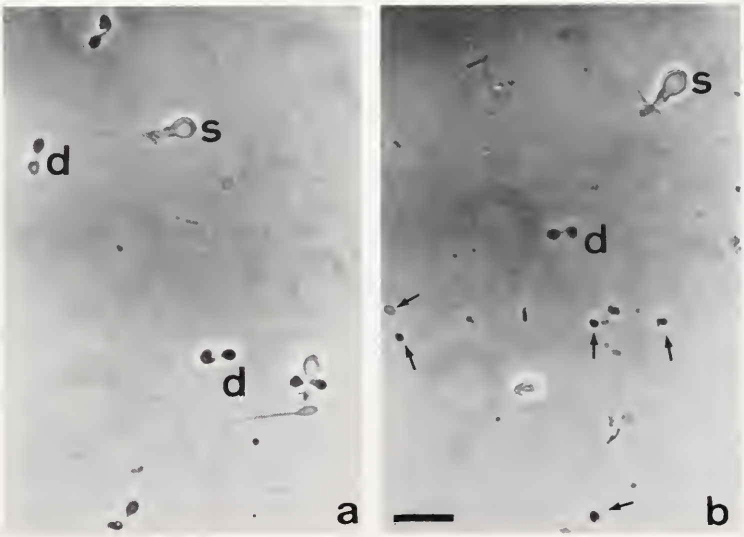

FIGURE 5. Photomicrographs under a phase contrast optics. The nematocyst capsules were obtained by dissolving hydras in 2% SDS solution. a) Control nematocysts. b) Those obtained from hydras treated by 2 m*M* PG for 75 h. In (b), many small objects indicated with arrows are, presumably, desmoneme capsules degrading (s, stenotele; d, desmonemes). Scale bar equals 20 μm.

PG is a specific irreversible inhibitor of several enzymes, including γ-cystathionase (Washtien and Abeles, 1977) and aspartete aminotransferase (Tanase and Morino, 1976). Among these enzymes, γ-cystathionase, which catalyzes the final step of L-cysteine biosynthesis, is most strongly inhibited by irreversible binding of PG at the active site. We examined the antagonistic effect of L-cysteine. At low concentration (below 5 m*M*), L-cysteine partly repressed the effect of 2 m*M* PG on hydra. At higher concentrations (above 5 m*M*), it was toxic to the hydras. Therefore, its antagonistic effects could not be examined.

Three methods have been used to obtain hydras lacking some types of nematocytes. The first is chemical treatment, for example, with colchicine (Campbell, 1976) or hydroxyurea (Bode *et al.*, 1976). These treatments, however, also deplete the interstitial cells and nerve cells. The second is the genetic method of isolating mutants by inbreeding. Sugiyama and Fujisawa (1977) have obtained many mutant hydras, but no hydra which lack nematocytes only. Such a strain would be difficult to culture and propagate, since such hydras could not capture prey. However, one strain which possesses no stenoteles on the tentacles (Fujisawa and Sugiyama, 1978) has been isolated. The third method, electrical stimulation, causes discharge of most of the stenoteles, but not most other nematocysts, especially desmonemes (Zumstein, 1973).

Compared with the above three methods, PG treatment is an easy way to obtain many hydras with normal interstitial and nerve cells, but lacking nematocytes, especially desmonemes and A. isorhizas.

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LITERATURE CITED

BODE, H. R., AND C. N. DAVID. 1978. Regulation of a multipotent stem cell, the interstitial cell of hydra. *Prog. Biophys. Molec. Biol.* **33:** 189–206.

BODE, H. R., AND K. M. FLICK. 1976. Distribution and dynamic of nematocyte population in *Hydra attenuata. J. Cell. Sci.* **21:** 15–34.

BODE, H. R., K. M. FLICK, AND P. M. BODE. 1977. Constraints on the relative sizes of the cell population in *Hydra attenuata. J. Cell. Sci.* **24:** 31–50.

BODE, H. R., K. M. FLICK, AND G. S. SMITH. 1976. Regulation of interstitial cell differentiation in *Hydra attenuata.* I) Homeostatic control of interstitial cell population size. *J. Cell Sci.* **20:** 29–46.

CAMPBELL, R. D. 1967. Tissue dynamic of steady state growth in *Hydra littoralis.* III) Behavior of specific cell type during tissue movement. *J. Exp. Zool.* **164:** 379–392.

CAMPBELL, R. D. 1976. Elimination of hydra interstitial cell and nerve cells by means of colchicine. *J. Cell Sci.* **21:** 1–13.

DAVID, C. N. 1973. A quantitative method for maceration of hydra tissue. *Wilhelm Roux' Archiv.* **171:** 259–268.

DAVID, C. N., AND D. CHALLONER. 1974. Distribution of interstitial cells and differentiating nematocytes in nests in *Hydra attenuata. Amer. Zool.* **14:** 537–542.

DAVID, C. N., AND A. GIERER. 1974. Cell cycle kinetics and development of *Hydra attenuata.* III) Nerve and nematocyte differentiation. *J. Cell Sci.* **16:** 359–372.

FUJISAWA, T., AND T. SUGIYAMA. 1978. Genetic analysis of developmental mechanism in *Hydra.* IV) Characterization of a nematocyst deficient strain. *J. Cell Sci.* **30:** 175–185.

HIRAKAWA, Y., AND H. KIJIMA. 1980. Behavioral analysis of glutathione receptor of *Hydra. J. Comp. Physiol. A.* **135:** 73–81.

MARISCAL, R. N. 1974. Nematocysts. In: *Coelenterate Biology.* Muscatine, L. and H. M. Lenhoff, Eds. Academic Press: New York, Pp. 129–178.

SUGIYAMA, T., AND T. FUJISAWA. 1977. Genetic analysis of developmental mechanism in *Hydra.* I) Sexual reproduction of *Hydra magnipapillata* and isolation of mutant. *Develop. Growth Differ.* **19:** 187–200.

TANASE, S., AND Y. MORINO. 1976. Irreversible inactivation of aspartate aminotransferases during transamination with L-propargylglycine. *Biochem. Biophys. Res. Commun.* **64:** 1301–1308.

WASHTIEN, W., AND R. H. ABELES. 1977. Mechanism of inactivation of γ-cystathionase by the acetylenic substrates analogue propargylglycine. *Biochemistry* **16:** 2485–2491.

ZUMSTEIN, Z. 1973. Regulation der Nematocyten—Produktion bei *Hydra attenuata* Pall. *Wilhelm Roux' Archiv.* **173:** 249–318.

Reference: *Biol. Bull.* **161:** 402–415. (December, 1981)

CRYSTALLINE AXES OF THE SPINE AND TEST OF THE SEA URCHIN *STRONGYLOCENTROTUS PURPURATUS*: DETERMINATION BY CRYSTAL ETCHING AND DECORATION

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ABSTRACT

By means of crystal etching and scanning electron microscopy the c- and a-axes of skeletal units of *Strongylocentrotus purpuratus* have been determined. Crystal etching and crystal decoration were found to be equally useful in demonstrating that the spines are single crystals of magnesian calcite. All regions of spine cross sections had common c- and a-axes. Comparisons of etched figures to decorated crystals on the same cross section of a spine showed that the grooves of the etch figures were parallel to the edges of the upper faces of the decorated crystals. The a-axes of the primary spines differed from those of the interambulacral plates to which they were attached.

Certain regions of the tubercle were shown by SEM examination of decorated tests to be polycrystalline aggregates lacking uniform crystal orientation, whereas the remaining portions of the interambulacral plates shared the same a-axes. The primary plates and demiplates of the ambulacral plates were each single crystals whose a-axis, and often c-axis, orientations were independent of adjacent compartments.

Reflective properties of decorated plates coated with gold-palladium have been shown to be useful in demonstrating differences in crystal orientation between adjacent interambulacral plates and between compartments of individual ambulacral plates.

INTRODUCTION

The skeleton of the Echinoidea is of crystallographic interest because of its ultrastructure and the orientation of its units. The spines and plates are magnesian calcite formed as a mineral meshwork of smooth trabeculae by cells of the dermis within the interconnecting spaces (Weber *et al.*, 1969; Heatfield, 1971; Märkel, 1981). X-ray diffraction and polarized light studies have indicated that the spines and plates, with the exception of tubercles which bear spines, are single crystals (Schmidt, 1930, 1932; West, 1937; Donnay, 1956; Raup, 1962, 1966; Currey and Nichols, 1967; Donnay and Pawson, 1969; Nissen, 1969).

By means of polarization microscopy, it has been shown that in spines the c-axis is parallel to the axis of elongation (Schmidt, 1930, 1932; West, 1937; Raup, 1962, 1966) and that in the ambulacral and interambulacral plates of *Strongylocentrotus purpuratus* the c-axis is normal to the outer surface, an orientation common to many, but not all, species (Raup, 1962). Other crystal axes were not determined in these studies since the optical method is limited to defining the

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direction of the c-axis. X-ray diffraction, which can resolve the c-axis and the a-axes, has been applied to the analysis of echinoid skeletons in only a limited way and has been directed primarily to the question as to whether the spines and plates constitute single crystals (Garrido and Blanco, 1947; Donnay, 1956; Donnay and Pawson, 1969).

The method of crystal decoration has proved valuable in the crystallographic analysis of larval and adult echinoid skeletons (Okazaki and Inoué, 1976; Okazaki *et al.*, 1980; Dillaman and Hart, 1981) as well as calcareous sponge spicules (Jones, 1955) and the test of a foraminiferan (Towe, 1977). In this method, skeletal structures placed in a saturated solution of $CaCO_3$ become decorated with calcite crystals whose orientation indicates the orientation of the crystals on which they form. Observations of the decorated crystals by electron microscopy permits determination of the c- and a-axes (Dillaman and Hart, 1981).

The view that the plates of the echinoderm test are single crystals has not found universal acceptance. Based on evidence from polarized light and observation of surface replicas by transmission electron microscopy, Towe (1967) suggested that interambulacral plates, which appear to be single crystals, are formed by oriented polycrystalline growth followed by a maturation involving recrystallization and continuous fusion and coalescence. The evidence presented does not include information on the a-axes of the skeletal elements and for this reason cannot completely define crystal orientation. By means of Laue X-ray diffraction and crystal decoration, Dillaman and Hart (1981) were able to show that the interambulacral plates conform to the criteria of single crystals as indicated by c- and a-axis orientation. Further, examination of the ultrastructure of the fenestrated plates by scanning electron microscopy gave no evidence of discontinuities or a polycrystalline structure.

In the present study we have carried out further detailed analysis of the skeletal structure of *Strongylocentrotus purpuratus* by the method of crystal etching. It appeared possible that by producing etch figures the crystal planes of skeletal units might be made evident and so permit the determination of c- and a-axes and crystal orientation. This has proved to be the case. Results obtained by crystal etching were found to give results equivalent to those by crystal decoration. Then, using decoration, we compared crystal orientation of plates and attached spines and the orientation of the crystal compartments within single ambulacral plates of the test.

MATERIALS AND METHODS

Specimens of adult *Strongylocentrotus purpuratus* obtained from Pacific Biomarine Laboratories, Venice, California were held in large recirculating sea water tanks at room temperature. Samples of primary spines, ambulacral and interambulacral plates were prepared by removing Aristotle's lantern and internal organs from living urchins and immersing the remaining skeletal material and attached soft tissue in Clorox (5.25% sodium hypochlorite) for 5 hours. This treatment removed all organic material covering and connecting the skeletal components. After 5 washes with distilled water adjusted to pH 8.0 with NaOH, primary spines and coronal plates were dehydrated through 70%, 95% and 100% ethanol and stored in 100% ethanol.

Etching of spines and plates

For etching of skeletal elements, Clorox-treated spines and plates were returned to distilled water, pH 8.0, by hydration through 95% and 70% ethanol. Entire spines and those fractured at right angles to the long axis were then immersed in 1–3% acetic acid (pH 2.6–2.8) for 4–10 minutes. Coronal plates were disarticulated and treated with 0.1–0.01% acetic acid (pH 3.2–3.7) for 10 minutes. Etching was stopped by transfer to 0.01 *N* NaOH, followed by repeated washes in distilled water, pH 8.0.

Decoration of spines and plates

Etched spines and plates were decorated with calcite crystals following the method of Okazaki *et al.* (1980). Samples in distilled water were transferred into a 0.1 *M* $NaHCO_3$ solution to which was added a 0.1 *M* $CaCl_2$ solution to give a ratio of the two solutions of 5:2. After 5 minutes in the final mixture, specimens were washed with distilled water, pH 8.0, and dehydrated through an ascending series of ethanol.

Scanning electron microscopy

Untreated, etched, and etched and decorated spines and plates after dehydration with ethanol were air-dried and attached to aluminum stubs with Duco cement. After sputter-coating with gold-palladium, specimens were examined with a Philips 501 scanning electron microscope at 15 or 30 KV.

RESULTS

1. Structure of untreated coronal plates and spines.

Coronal plates. The corona consists of 20 curved rows of plates, five ambulacral areas of two-plate rows alternating with five interambulacral two-plate rows (Fig. 1A). Each plate bears one large primary tubercle on which the primary spine is situated. There are several secondary tubercles with spines surrounding the primary tubercle (Figs. 1D, 6A, B). All tubercles consist of a basal portion (the boss) having the shape of a low truncated cone which is surmounted by a terminal knob (the mamelon) lying on a narrow shoulder. The boss is encircled by a bare area to which are attached the muscles operating the spine. These four parts of the tubercle are designated as regions 1, 2, 3, and 4 (Fig. 6C).

Each ambulacral plate is an assembly of seven compartments, each pierced by seven paired pores for the passage of the podia (Hyman, 1955). These compartments consist of two primary plates (Fig. 6A, a, g) and five demiplates inserted between them (Fig. 6A, b, c, d, e, f). Every part of the coronal plates exhibits a fenestrated structure with the exception of the outer surface of the mamelon, which is solid (Figs. 6A, B, and C). Under the scanning electron microscope, surfaces of the coronal plates and spines appear smooth and do not display any surface texture suggestive of their crystalline composition, as pointed out by Currey and Nichols (1967) and Dillaman and Hart (1981).

Primary spines. The spines of *Strongylocentrotus purpuratus* have been described in detail by Heatfield (1971). The main regions are a basal portion, a milled ring, and a tapering shaft (Fig. 1B, C). The concave surface of the base and the mamelon of the tubercle of the associate plate form a ball-and-socket joint (Fig. 1C).

In transverse section, the spine is characterized by a series of calcite rings (Fig. 2A) in a continuous meshwork. In the outer zone, wedges of solid calcite alternate with the meshwork to form radii and concentric rings. The outermost surfaces of

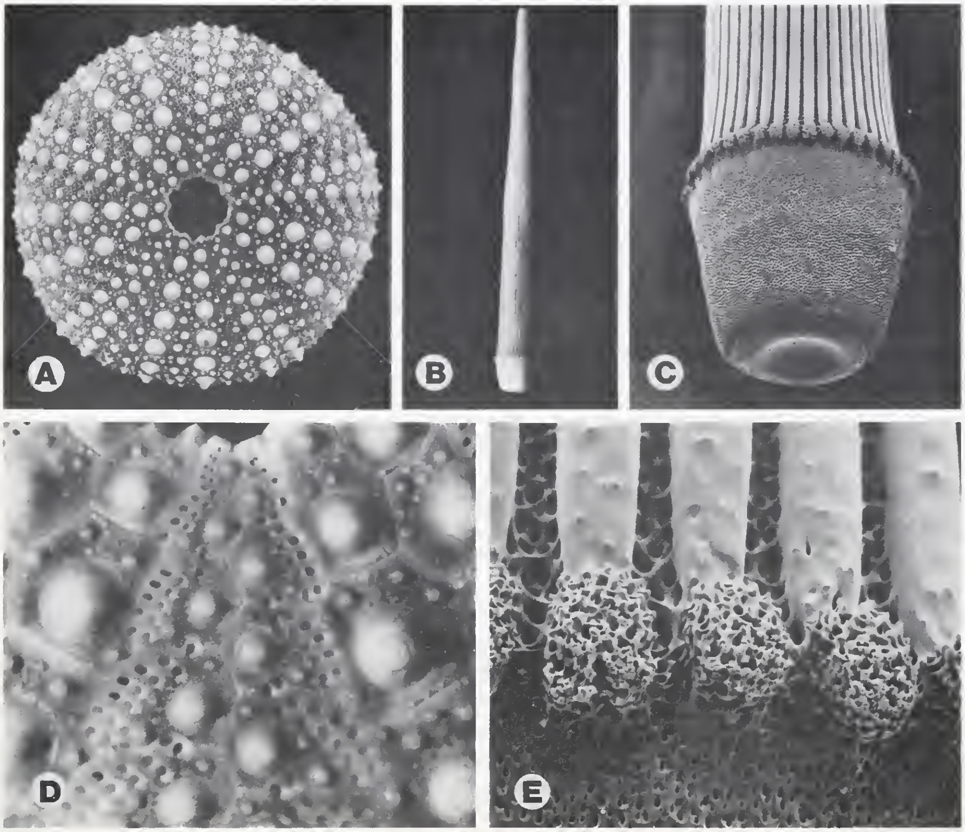

FIGURE 1. A. Light micrograph of Clorox-treated test showing curved rows of plates, ×2. B. Light micrograph of spine showing the basal portion, milled ring and tapered shaft, ×5. C. Scanning electron micrograph of spine showing concave base, ×25. D. Light micrograph of test showing double row of ambulacral plates, each pierced by seven pairs of pores. Also apparent are the mamelons that accept the spine, ×10. E. Scanning electron micrograph of base of spine showing trabecular components between the longitudinal columns, ×210.

the wedges are smooth and rounded and appear as longitudinal columns in side view (Fig. 1C, E). The number of columns on a single mature spine ranged from 40 to 60, and the number frequently increased from the milled ring to the tip of a spine. In transverse section, the increase was seen as new radii (Fig. 2A). In the present study, the wedges forming the columns were loci for observations of etch figures and decorated crystals.

2. *Etch figures and decorated crystals on the spine.*

Etch figures created by treating spines with dilute acid are shown in Figures 2B, C, D. Figure 2B is a lateral view showing identical cleavage directions on the two columns when viewed from the same perspective. Figures 2C, D show etch figures of two wedges on a cross section of a spine located in regions I and II, respectively, of Figure 2A. On each wedge, three cleavage faces are evident. The faces are parallel in the two wedges even though they occupy different positions

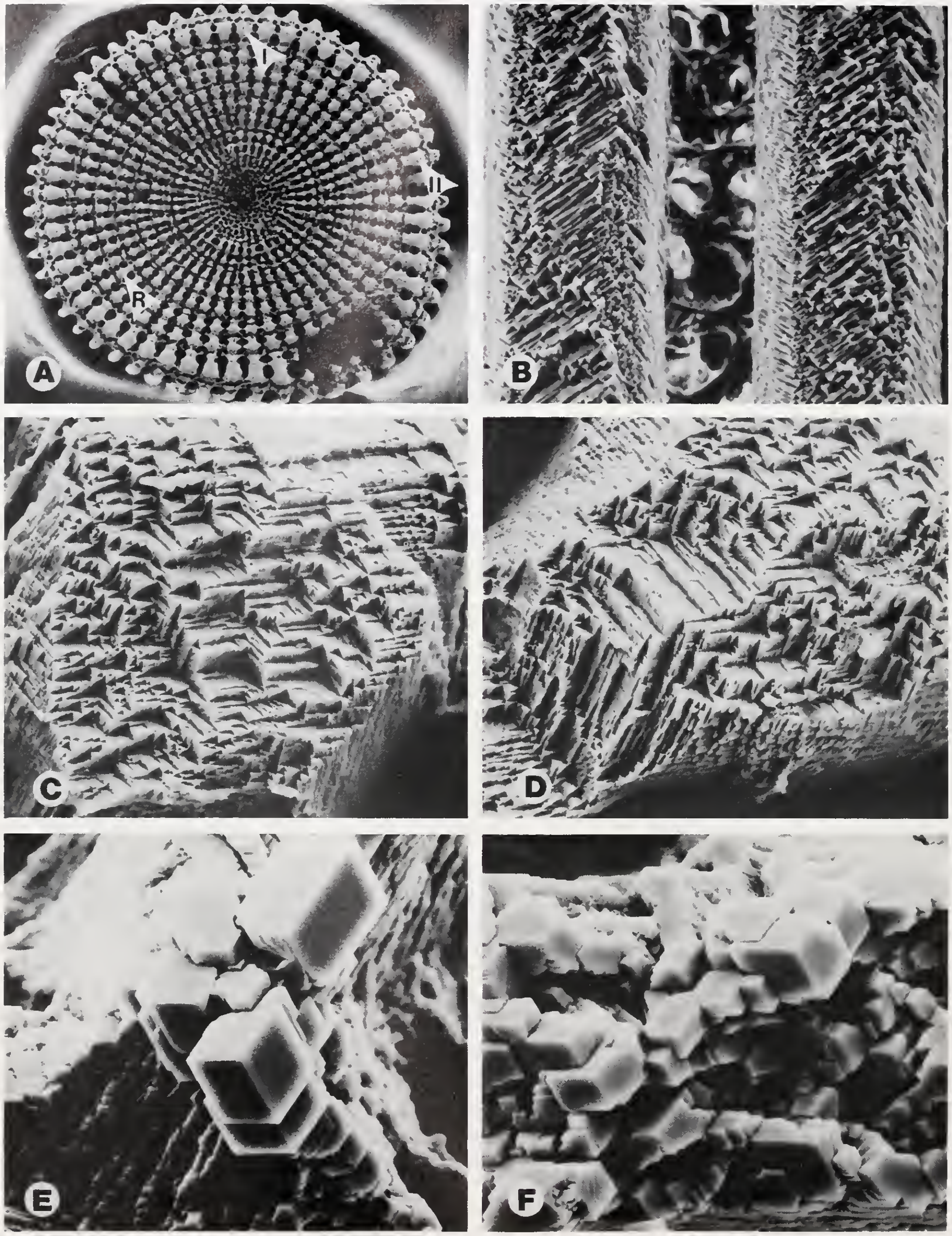

FIGURE 2. Scanning electron micrographs of spines. A. Transverse section of spine showing series of rings. Also note new radius (R) that serves to increase the circumference, ×41. B. Longitudinal column after etching with acetic acid. Note identical cleavage directions in adjacent columns, ×350. C. and D. Etch patterns on surface of transverse section from regions I and II of Fig. 2A. Note that cleavage angles are identical, ×1250. E. and F. Decorated crystals on surface of transverse section at sites comparable to I and II in Fig. 2A. Note that crystal edges are parallel, ×2500.

on the circumference of the spine. Each wedge of the spine shows the same orientation. Since each of the three angles formed by the intersection of the three cleavage faces were 120° it was judged that the observations were made parallel to the electron beam along the c-axis of the spine. One can accordingly conclude from the correspondence of the angles that the spine is a single crystal whose c-axis is normal to the cross-section, that is, parallel to the axis of elongation of the spine. Further, since the angle between the a-axis and the ridge of calcite is 30° when viewed along the c-axis (Fig. 3), etch figures can also be used to determine the a-axes. From etch figures in Figures 2C, D, the three a-axes of Figure 2A were determined and are indicated by the superimposed dark lines of Figure 3.

Because the etch figures were relatively small, it was necessary to make many measurements from a single micrograph for exact determinations of cleavage surfaces. By decorating the etched surfaces, determinations of c- and a-axes were greatly facilitated. The rhombohedral decorated calcite crystals were of sufficient size and the coigns prominent to the extent that the stage of the electron microscope could be oriented so that the ridges of single crystals formed 120° angles with one another. Thus, one could assume that the crystal was being observed along its c-axis. The a-axes could then be calculated. Figures 2E, F are representative of the habits of the crystals as observed at all locations on the cross section of a single spine, including its base. This result supports the conclusion based on etch figures that the spine is a single crystal. Although calcite crystals could be grown on unetched surfaces of the spine, crystals of varying orientation frequently developed, presumably from small chips produced by cutting which served as nuclei.

To test the relationship between etch figures and decorated crystals, individual spines were first etched and half of the spine then decorated, permitting a comparison of etch and decorated figures on the same spine (Fig. 4A, B). An examination of these figures shows that the grooves of the etch figures precisely paralleled the ridges of the decorated crystals. However, as Figure 3 indicates, the ridges on

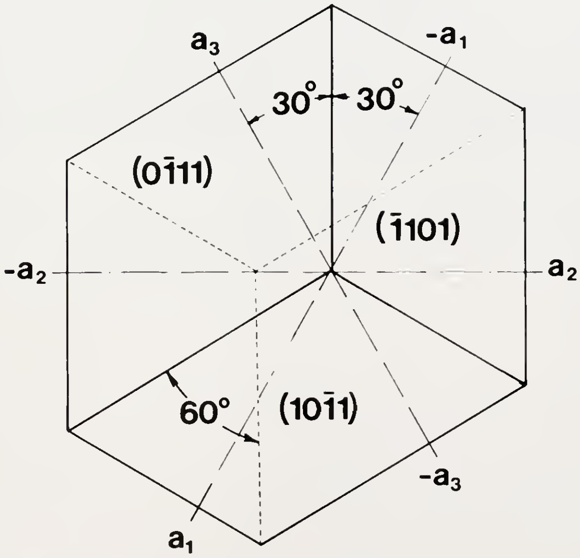

FIGURE 3. Schematic representation of calcite rhombohedron viewed along c-axis. Bold lines represent the upper edges of the rhomb while dotted lines indicate lower edges.

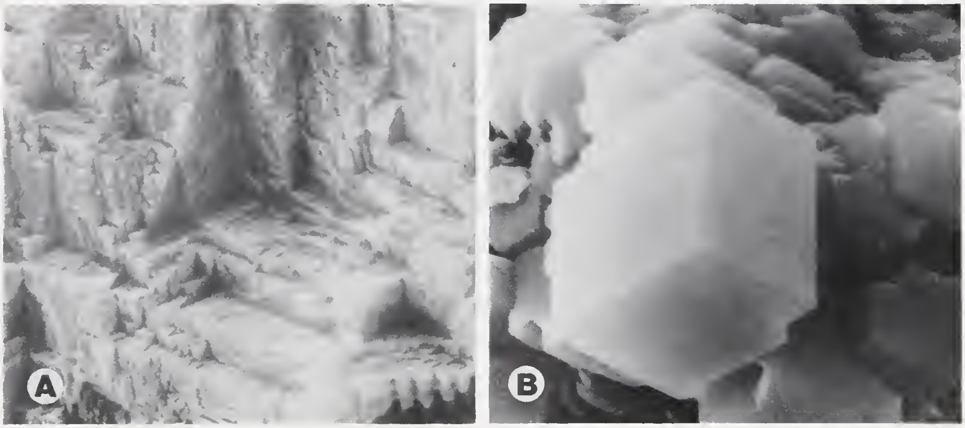

FIGURE 4. Transverse section of a single spine viewed with the scanning electron microscope after etching (A) and after etching and decoration (B). Note that grooves of etch figures parallel the edges of the decorated crystals. A., ×11,000, B., ×5000.

the front form angles of 60° with the ridges on the rear of the calcite rhombohedron when viewed along the c-axis (*cf.* solid lines and dotted lines). Since one would initially assume that the etch figure would represent the removal of calcite along cleavage faces, it was therefore necessary to provide an alternative explanation for the relationship between the etch and decorated figures. As an approach to a possible explanation, mineral calcite whose initial crystal habit was evident was treated in exactly the same manner as the spine and then examined with the scanning electron microscope. When the specimen was observed along its c-axis as determined by its preexisting cleavage plane (Fig. 5A), two types of etch figures were observed, as shown diagrammatically in Figures 5B and C. Scanning electron micrographs of actual pits are shown in Figures 5E and F. Figures 5B and E correspond to a depression due to the removal of a rhombohedral sector, which was characterized by sharp grooves and smooth etch pit walls. Equally frequent were etch figures shown in Figures 5C and F with grooves that paralleled the ridges of the underlying crystal (Fig. 5A). The two lateral walls showed a stepped appearance (Fig. 5F) whereas the third wall was smooth. As a consequence, the groove formed where the two stepped walls met was not as sharp as in the previously mentioned type of etch figure. In both types, however, the grooves formed 120° angles with one another when viewed along the c-axis, demonstrating they were equally capable of revealing the c-axis of the crystal. Irrespective of underlying etch figures, orientations of the edges of the decorated crystals were all the same as the initial calcite (Fig. 5D).

3. Decorated interambulacral and ambulacral plates.

Interambulacral plates. Etched and decorated interambulacral plates (Fig. 6B) examined by scanning electron microscopy showed several patterns of the decorated crystals. On the tip of the tubercle (Fig. 6C, region 1) the crystals were arranged in sectors of varying orientation (Fig. 6E). The individual sectors varied in both their c- and a-axes and consequently represented coarse polycrystalline aggregates. Region 2 of the tubercle (Fig. 6C) was also made up of polycrystalline aggregates. This region of the skeleton was less dense than region 1 due to its more open

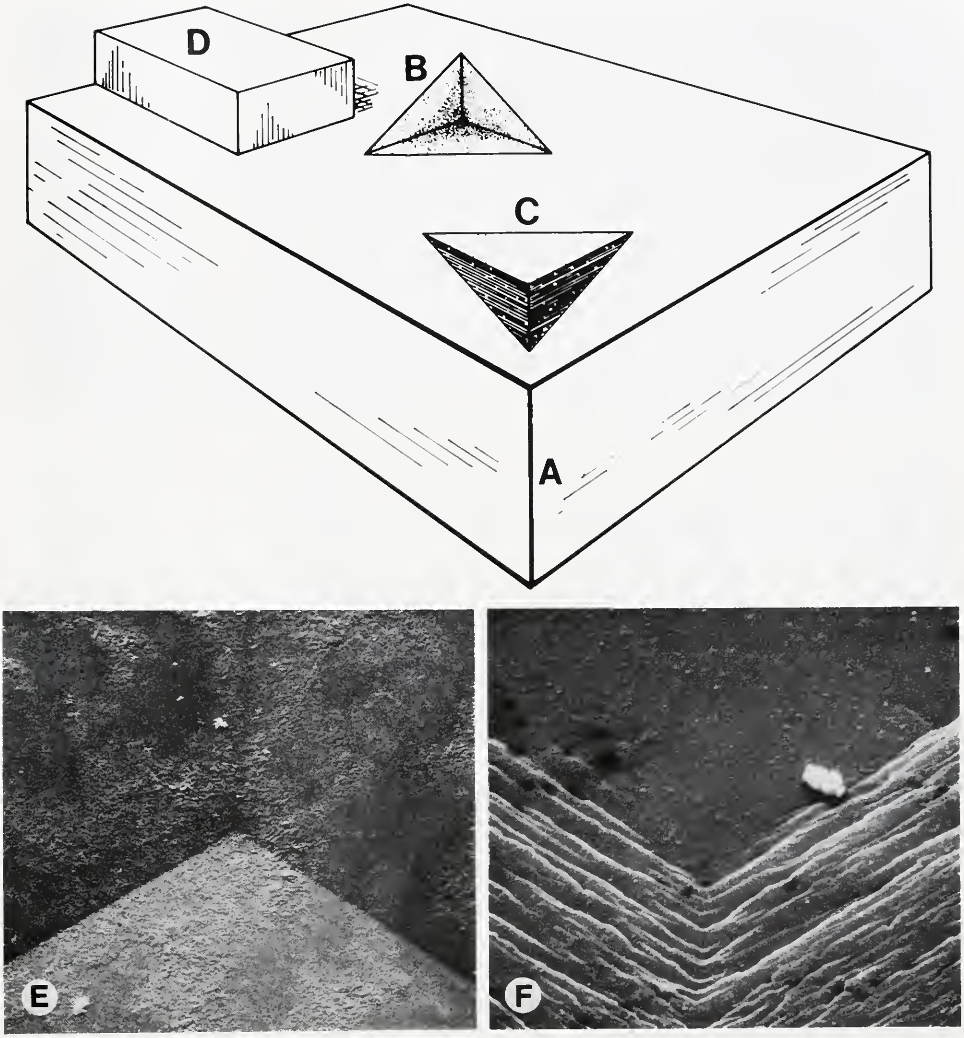

FIGURE 5. Schematic representation of mineral calcite etched and decorated. Cleavage face of native crystal (A) and decorated crystal (D) can be compared with etch pits with smooth sides (B) and stepped sides (C). E. and F. are scanning electron micrographs of etch pits such as indicated in B. and C., respectively. E., ×1900, F., ×2750.

trabecular structure (Fig. 6F). Crystal sectors in region 2 also showed varying c- and a-axes. In regions 3 and 4 of the tubercle (Fig. 6C) and remaining parts of the plate, the decorated crystals were especially large at the growing tips of the trabeculae, and had ridges which were clearly parallel (Fig. 6D). Orientation of the sample so as to form 120° with all the ridges made it possible to determine that the c-axis was approximately perpendicular to the surface of the plate and further permitted calculation of the a-axes. The relationship between the plate margins (exclusive of the margin with the ambulacral plates) was examined. The difference in angle between the a-axes and the sides ranged from 5.9° to 9.5° with a mean value for 24 observations of 8.1° ± 5.0°. The a-axis directions were found

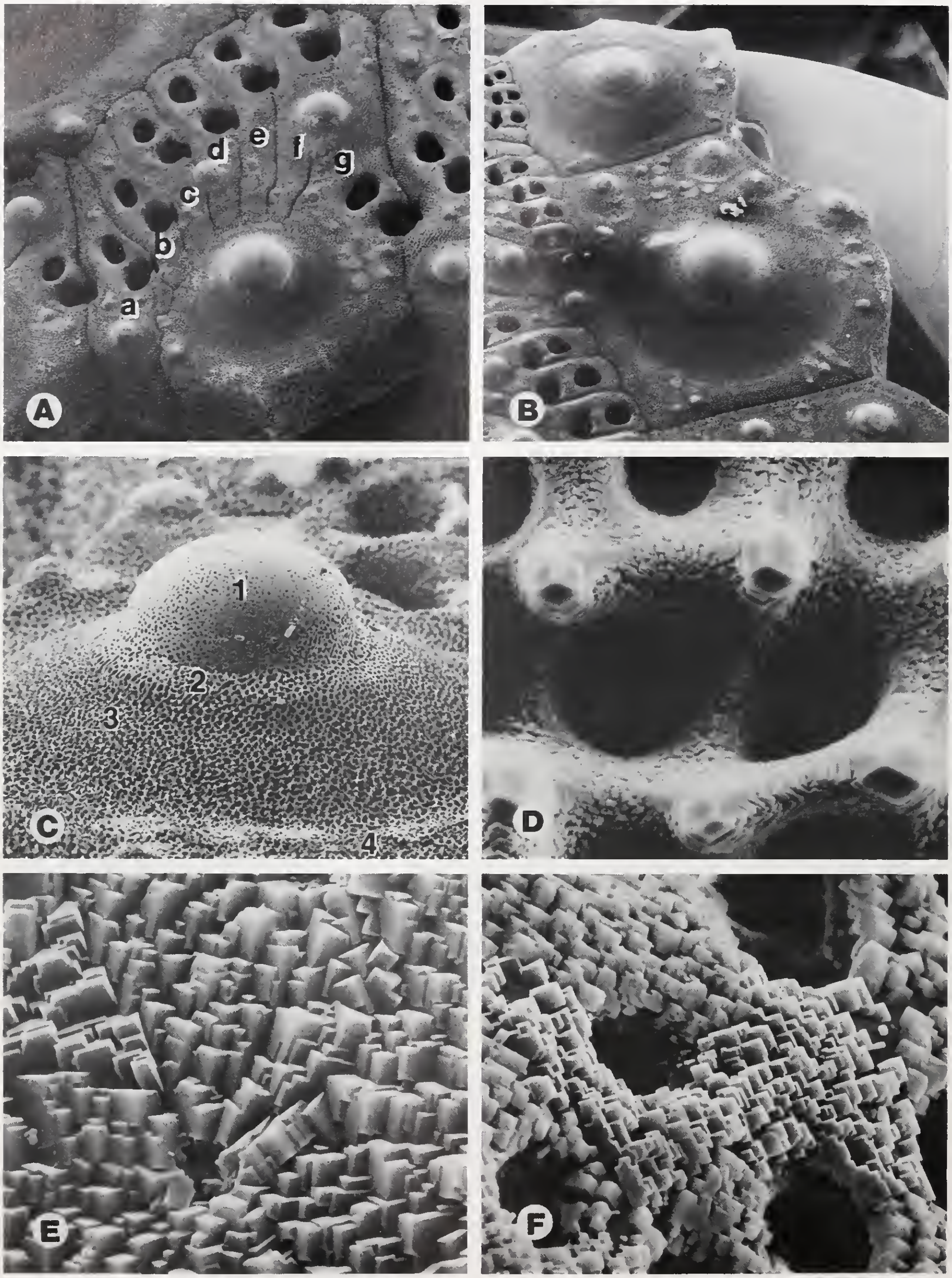

FIGURE 6. Scanning electron micrographs of plates of the test. A. Ambulacral plate showing two primary plates (a and g) and five demiplates (b through f), ×40. B. Interambulacral plates showing location of primary tubercle, ×20. C. Primary tubercle showing terminal knob (1), boss (2 and 3), and underlying plate (4), ×100. D. Decorated interambulacral plate showing large crystal at the growing tips of the trabeculae typical of regions 3 and 4 of Fig. 6C, ×1250. E. Decorated crystals in region 1 of Fig. 6C. Note varying orientation of crystal edges, ×2500. F. Decorated crystals from region 2 of Fig. 6C., ×2000.

to vary in relation to plate margins by Donnay and Pawson (1969) by X-ray diffraction and by Dillaman and Hart (1981) by crystal decoration.

By marking the edge of a spine and tubercle while they were connected and then decorating both, it was possible to compare the a-axes of an interambulacral plate to its primary spine. Figures 7A, B are representative of such preparations. The dark lines indicate their common side. Figures 7C, D correspond to decorated crystals on the plate and spine, respectively. In five such preparations, none indicated that the plate and spine shared the same a-axes.

Ambulacral plates. Scanning electron microscopic examination of etched and decorated ambulacral plates (Fig. 6A) indicated that often the c- and a-axes were different among the seven compartments of a single plate. Measurements of the angles formed by the ridges of individual compartments of two ambulacral plates are given in Table I. Letters a through g refer to compartments as labelled in Figure 6A. In both plates, compartment e was chosen as reference. That is, each plate was rotated so that compartment e was viewed along its c-axis, and then the

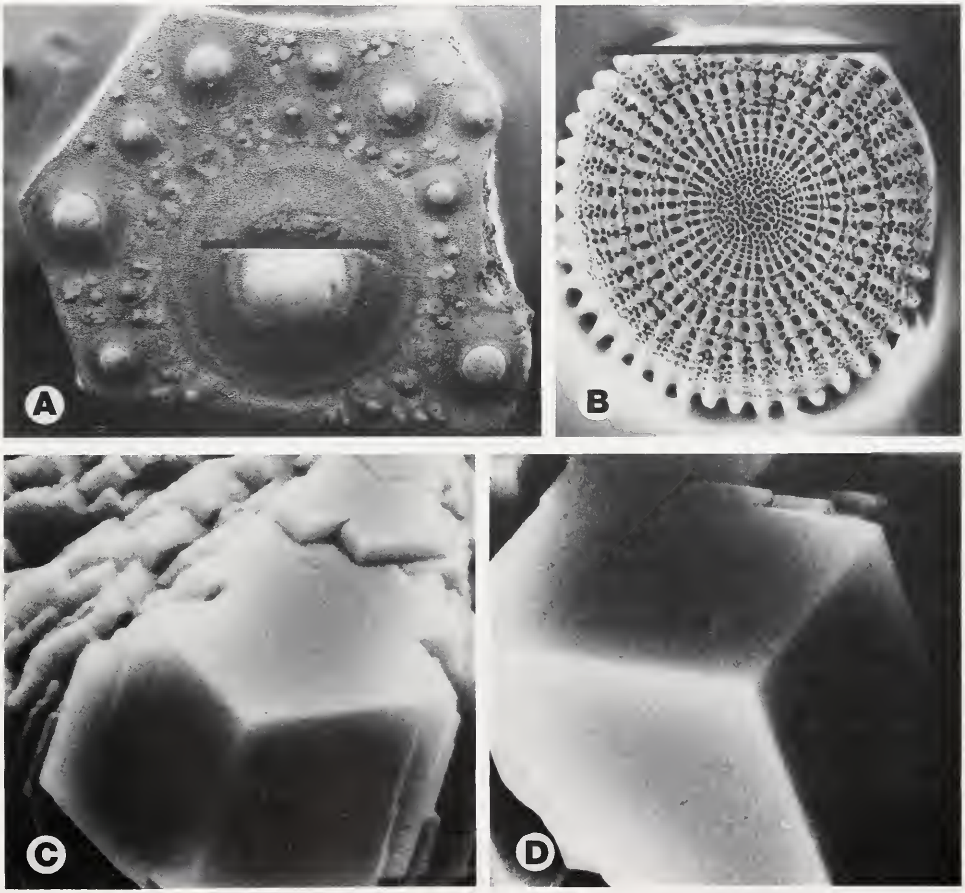

FIGURE 7. Scanning electron micrographs of the tubercle of interambulacral plate (A) and a transverse section of its primary spine (B), each having been flattened on one side to indicate common alignment (Bars). C. and D. are decorated crystals from areas shown in A. and B., respectively. A., ×22, B., ×66, C., and D., ×11,000.

TABLE I

Angles of three cleavage faces surrounding c-axis

	Symbol of compartment in ambulacral plate (see Fig. 6 A)						
Sample No.	a	b	c	d	e	f	g
1	180° 87 93	118° 129 113	120° 124 116	123° 122 115	120° 120 120	126° 118 116	129° 116 115
2	120 120 120	120 120 120	127 129 104	120 120 120	120 120 120	120 120 120	120 120 120
Shift of a-axis from e	+13	+20	—*	+2	0	−40	−21

*A shift of the a-axis for compartment c could not be measured in that the three angles of reference were not 120°. +, clockwise. −, counterclockwise.

adjacent compartments were viewed with only X and Y translation of the sample. In sample 1 of Table I, no other compartments shared the same c-axis with compartment e, and their deviation was quite varied. In sample 2, six of the seven compartments shared the same c-axis, compartment c being the only one showing a difference. However, when the a-axes of the six compartments of this sample were calculated, it was found that no two had the same orientation. The a-axes ranged from −40° to +20° relative to that of compartment e (Table I, bottom line). Clearly, each compartment of the ambulacral plate was a single crystal with crystal orientation (c- and a-axes) independent of its adjacent compartment.

4. *Light reflective properties of decorated and coated skeletal elements.*

The thin coat of gold-palladium on the surfaces of decorated spines and coronal plates serving as a conductive layer for scanning electron microscopy effectively made front-surface mirrors of the outer facets (0111, 1101, and 1011) of the calcite crystals. The result was that parallel facets within a given region acted as a single reflecting surface. Figure 8A shows the upper half of a test which was etched, decorated, coated with gold-palladium and illuminated with two flood lamps approximately 45° to the test. Certain interambulacral plates appear very bright while others are dark. The individual areas of ambulacral plates consisting of two primary and five demiplates also exhibited differences in their reflective properties. In Figure 8B, center, two rows of ambulacral plates are shown. In the left row, the top three plates do not reflect, and, in the last one, a single demiplate is bright. In the right row, a variety of reflective patterns is seen. The primary plates, second from top, are bright, and the demiplates are dark. The remaining two plates in the row have a mixed pattern with individual compartments varying from very bright through an intermediate series of grays to dark. Clearly, these results demonstrate that both the interambulacral and ambulacral plates may differ from adjoining plates in their crystal orientation. Within individual ambulacral plates, differences in orientation between compartments were also evident, as analysis of decorated crystals had shown.

DISCUSSION

The methods of etching and crystal decoration coupled with scanning electron microscopy used in the present study have provided information about underlying

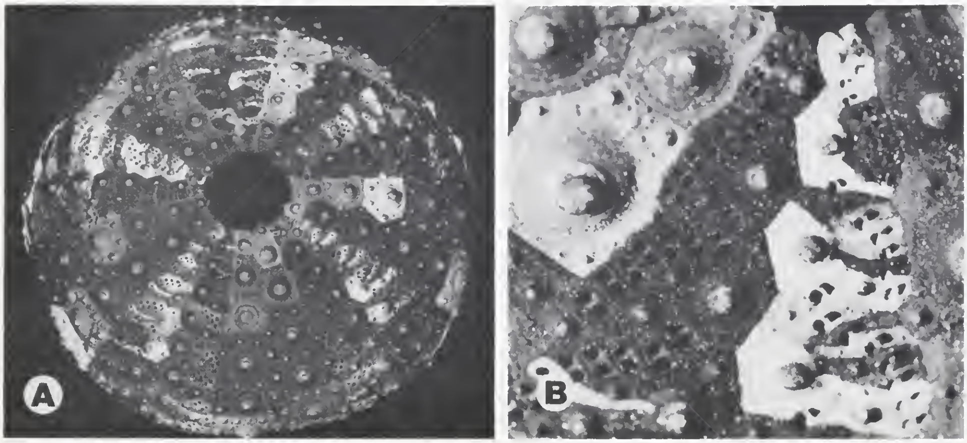

FIGURE 8. Light reflective patterns of test decorated and then coated with gold-palladium. A., ×3, B., ×15.

crystal faces and their relation to the macroscopic and ultrastructure of skeletal units of *Strongylocentrotus purpuratus*. Decoration of etched spines followed by examination with scanning electron microscopy facilitated the determination of a-axes in that the angles of the coigns of the decorated crystals were distinct and more easily measured than etch figures. In general, however, results from etching must be given more weight in that they represent the native crystal faces rather than a derivative. Etching and crystal decoration both showed the spines to be single crystals in that all regions of their cross sections and fine structure had common c- and a-axes.

When etch figures and decorated crystals were compared on the same spine, the grooves of the etch figures and the crystal ridges were parallel. Further, identical treatment of large crystals of mineral calcite showed that decorated crystals reliably reflect c- and a-axes. Etching of these crystals without subsequent decorations gave two types of etch figures, one which could be attributed to removal of unit cells and another parallel to the crystal margins and exhibiting stepping seen in "hopper crystals" (Buerger, 1970). One explanation of "hopper crystals" proposes that the stepping results from a change in the chemical environment of the crystal as it grows (Buerger, 1970). Within the internal environment of the spines, the magnesium and organic material, including pigment, could conceivably change as a result of the activity of the mineralizing cells and so give local changes within the crystal structure. With preferential etching of loci differing in lattice structure, such a stepped figure might be formed. Whatever the reason, it is of interest that mineral calcite with its lower magnesium content and absence of organic material formed two types of etch figures whereas only the stepped pattern was formed in the spines. Regardless of the type of etch figure encountered, the observation that decorated crystals reflect the same c-axis and a-axes as the underlying crystal as shown by etching indicates that decoration is a useful method for determining crystal orientation of sea urchin skeletal parts.

By etching with dilute acetic acid, it has been possible to confirm the polarized light microscopic observations of Raup (1959) showing that the primary spines of *Strongylocentrotus purpuratus* have their c-axis parallel to the axis of elongation. Since the a-axes can be determined from the three grooves of the etch figures, we could also confirm the conclusion of Donnay and Pawson (1969) from X-ray diffraction that the spine is a single crystal of calcite with a single c-axis and three a-axes.

We found that the a-axes of interambulacral plates and their primary spines did not correspond. One explanation is that the two structures develop independently with respect to their mineralization and with control limited to their c-axes which have the same orientation. A second explanation is that the base of the spine in rotating on the tubercle tip has a grinding action which breaks off small bits of the skeletal material (Donnay and Pawson, 1969). During growth, these bits might fuse to a polycrystalline structure as observed by Towe (1967) and in the present study (Fig. 6E, F).

Our limited analysis of ambulacral plate compartments showed wide diversity of crystal orientation favoring the view that each compartment arises separately. Of 14 plates examined (Table I), no two shared the same a-axes. This is noteworthy in light of the observation that in one plate six of seven compartments had the same c-axis. Analyses restricted to the c-axes would have indicated greater uniformity of crystal orientation than actually existed.

Diversity of orientation among ambulacral plates and compartments of single plates was strikingly evident in whole tests that had been etched, decorated, coated with gold-palladium, and then observed with directed visible light (Fig. 8 A, B). Although the small mean size of the crystal faces caused interference between adjacent reflective surfaces when viewed with a small diameter beam of visible light, this treatment was useful in making general assessments of crystal orientations. Differences that became evident by this simple method could then be analyzed precisely for c- and a-axis orientation using scanning electron microscopy.

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LITERATURE CITED

BUERGER, M. J. 1970. *Contemporary Crystallography.* McGraw-Hill, New York.

CURREY, J. D., AND D. NICHOLS. 1967. Absence of organic phase in echinoderm calcite. *Nature* **214:** 81–83.

DILLAMAN, R. M., AND H. V. HART. 1981. X-ray evaluation of a SEM technique for determining the crystallography of echinoid skeletons. *Scanning Electron Microscopy/1981* **III:** 313–320.

DONNAY, G. 1956. High-order transitions in mineralogy. *Yearb. Carnegie Inst.* **55:** 203–206.

DONNAY, G., AND D. L. PAWSON. 1969. X-ray diffraction studies of echinoderm plates. *Science* **166:** 1147–1150.

GARRIDO, J., AND J. BLANCO. 1947. Structure cristalline des pignanats d'oursin, *C, R. Acad. Sci. Paris* **224:** 485.

HEATFIELD, B. M. 1971. Growth of the calcareous skeleton during regeneration of spines of the sea urchin *Strongylocentrotus purpuratus* (Stimpson): a light and scanning electron microscope study. *J. Morph.* **134:** 57–90.

HYMAN, L. H. 1955. *The Invertebrates: Echinodermata. The Coelomata Bilateria,* Vol. 4. McGraw-Hill, New York.

JONES, W. C. 1955. Crystalline properties of spicules of *Leucosolenia complicata. J. Microsc. Sci.* **96:** 129–149.

MÄRKEL, K. 1981. Experimental morphology of coronar growth in regular echinoids. *Zoomorph.* **97:** 31–52.

NISSEN, H. U. 1969. Crystal orientation and plate structure in echinoid skeletal units. *Science* **166:** 1150–1152.

OKAZAKI, K., AND S. INOUÉ. 1976. Crystal property of the larval sea urchin spicule. *Devel. Growth and Differen.* **18:** 413–434.

Okazaki, K., K. McDonald, and S. Inoué. 1980. Sea urchin larval spicule observed with the scanning electron microscope. In: *The Mechanisms of Biomineralization in Animals and Plants.* M. Omori and N. Watabe Eds. Pp. 159–168.

Raup, D. M. 1959. Crystallography of echinoid calcite. *J. Geol.* **67:** 661–674.

Raup, D. M. 1962. Crystallographic data in echinoderm classification. *System. Zool.* **11:** 97–108.

Raup, D. M. 1966. The endoskeleton. In: *Physiology of Echinodermata.* R. A. Boolootian, Ed. Pp. 379–395.

Schmidt, W. J. 1930. Die Skeletstücke der Stachelhäuter als Biokristalle. *Zool. Jahrb. allgem. Zool.* **47:** 357–510.

Schmidt, W. J. 1932. Bestimmung der Lage der optischen Achse in Biokristallen. *Abderhalden's Handbuch der biol. Arbeitsmethoden,* Lief 289, Abt. 5, **2:** 1357–1400.

Towe, K. M. 1967. Echinoderm calcite: single crystal or polycrystalline aggregate. *Science* **157:** 1048–1050.

Towe, K. M. 1977. The crystallography of *Patellina corrugata* Williamson: a-axis preferred orientation. *J. Foram. Res.* **7:** 58–61.

Weber, J., R. Greer, B. Voight, E. White, and R. Roy. 1969. Unusual strength properties of echinoderm calcite related to structure. *J. Ultrastr. Res.* **26:** 355–366.

West, C. D. 1937. Note on the crystallography of the echinoderm skeleton. *J. Paleontol.* **11:** 458–459.

Reference: *Biol. Bull.* **161**: 416–425. (December, 1981)

α-METHYLGLUCOSIDE TRANSPORT BY THE GILL OF THE OYSTER *OSTREA EDULIS*[1]

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ABSTRACT

The glucose transport system in the gill of *Ostrea edulis* was studied using α-methyl[U-^{14}C] glucoside. The same mobile carrier transported α-methylglucoside (α-MG) and D-glucose in the isolated gill. Unlike D-glucose α-MG was not appreciably metabolized to CO_2. It was hypothesized that the mobile carrier is specific for the hydroxyl group at the C_3 position of D-glucose. Ouabain (0.1 mM) did not reduce α-MG uptake, thus suggesting that Na^+ transport was either via a ouabain insensitive pump or a ouabain sensitive pump inaccessible to ouabain dissolved in seawater, the external medium. Compounds capable of collapsing the transmembrane electrical potential reduced α-MG uptake. Naphthalene, a compound known to hyperpolarize muscle cells, stimulated α-MG uptake. Inner leaks via the mobile carrier were determined to be much greater than outer leaks. The results indicate that the glucose transport system in the oyster gill has many similarities to Na^+-dependent transport of glucose in mammalian small intestines and proximal renal tubules. Naphthalene stimulation of α-MG uptake is consistent with a previous study demonstrating naphthalene stimulation of glucose metabolism in gill tissue.

INTRODUCTION

The uptake of simple sugars by the bivalve gill was described by Péquignat (1973) and Bamford and Gingles (1974), who showed that D-glucose and D-galactose were rapidly taken up by the isolated gill of *Crassostrea gigas.* However, 3-0-methyl-D-glucose (3-MG) was not taken up. Glucose uptake depended on the external Na^+ concentration. Thus, Bamford and Gingles (1974) concluded that the mechanism of transport was probably a Na^+ -glucose co-transport system.

The glucose transport system in the gill removes dissolved glucose from seawater. Sources of naturally-occurring glucose include plant and animal excretions and decomposition (Vaccaro and Jannasch, 1966). Active uptake of dissolved organic molecules may provide a significant supplement to the nutrition of bivalves (Wright and Stephens, 1978). Additionally, active reabsorption may serve to counteract the leakage of metabolically important substrates from the intra and intercellular space.

The study of the glucose transport system is of interest from both a physiological and toxicological perspective. Membrane bound transport systems are important

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Abbreviations: 3-MG, 3-0-methyl-D-glucose; α-MG α-methylglucoside; DNP, dinitrophenol; CCCP, carbonylcyanide-M-chlorophenylhydrazone; PCP, pentachlorophenol; $[MG]_{in}$ $[MG]_{out}^{-1}$, concentration ratio of α-MG in tissue and seawater; K_i, inhibitor constant; R_f, mobility relative to the solvent front.

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control points of metabolism (Elbrink and Bihler, 1975; Weiner, 1979). The first site of interaction between chemical toxicants and living tissue is the cell membrane (Marchesi, 1978; Pritchard, 1979). Thus, membrane bound transport systems may serve as useful probes for studying the metabolic effects of lipophilic toxicants such as naphthalene. The glucose transport system is especially suited for such study since nonmetabolized analogs of glucose (*i.e.* methylglucosides) are available which allows the study of transport in isolation from subsequent metabolic transformation.

The present study more clearly defines the mechanism of glucose transport in the isolated gill of oysters, using the European flat oyster, *Ostrea edulis*, as a representative species. The specific objectives were: (1) identify a non-metabolized transport analog, (2) determine the effects of known metabolic inhibitors and naphthalene on transport, and (3) determine the effect of inhibitors on leakage.

MATERIALS AND METHODS

All analytical chemicals were at least reagent grade. D-[U-^{14}C]glucose (274 mCi mmol^{-1}), [^{3}H]inulin (1.9 Ci mmol^{-1}), [l-^{14}C]naphthalene (36 mCi mmol^{-1}), and α-methyl[U-^{14}C]glucoside (α-MG) (184 mCi mmol^{-1}) were obtained from the Amersham Corporation (Arlington Heights, Illinois). Dinitrophenol (DNP), D-glucose, α-methylglucoside (α-MG), and ouabain were obtained from Sigma Chemical Co.; carbonylcyanide-M-chlorophenylhydrazone (CCCP) from Aldrich; iodoacetate (Na$^+$-salt) and pentachlorophenol (PCP) from Baker Chemicals. Curve fitting was accomplished by regression analysis (Neter and Wasserman, 1974). The mathematical models were transformed to the appropriate linear form and then the best fit judged by visual inspection and the magnitude of the coefficient of determination, r^2.

Pre-conditioning of oysters

Oysters (*O. edulis*) obtained as cultchless spat from International Shellfish Enterprises, Inc., Moss Landing, California, were held in flowing seawater at the Oregon State University Marine Science Center at Newport, Oregon. When approximately 1 year old, they were transferred to a closed holding aquarium (Instant Ocean) and maintained at 28.5 ‰ and 15°C with no apparent source of nutrition for 6–10 days. All experiments were conducted at 15°C in air-saturated Millipore-filtered (0.22 μm) seawater. The gill and overlying mantle were removed immediately before each experiment. The isolated gill preparation was composed of all four intact demibranchs (ascending and descending lamellae) and the associated mantle tissue.

Metabolism of α-methylglucoside (α-MG)

The gill tissues from 8 oysters were placed into separate incubation vessels containing 40 ml of filtered seawater, 200 ppm streptomycin, 100 ppm chloramphenicol, and 300 ppm penicillin each. D-[U-^{14}C]glucose (2 μCi) was added to four of the vessels and α-[U-^{14}C]MG (2 μCi) added to the remaining four. Each vessel was sealed with a Teflon gasket and aerated; the effluent air from each vessel was bubbled through a CO_2 trap containing 5 ml of Oxisorb-CO_2 (New England Nuclear, Boston, Massachusetts). After 1 h the tissues were removed and digested in NCS tissue solubilizer (Amersham Corporation), the vessels were resealed, 1 ml of concentrated sulfuric acid was injected into each vessel via a septum, and aeration was resumed for 1 h. The contents of the CO_2 traps, samples of the seawater, and the tissue digests were assayed by standard liquid scintillation counting procedures on a Packard Tri-Carb LSC.

Uptake of α-MG

A tissue holder was constructed that held up to 50 tissues, each suspended on a separate stainless steel stylet. Groups of 3–10 tissues were suspended in this way in incubation vessels containing 200 ml of filtered seawater and [^{3}H]inulin. The ratio of seawater to tissue was always greater than 100:1. After 30–45 min of incubation in [^{3}H]inulin, the tissues were transferred simultaneously, on the tissue holders, to a fresh set of incubation vessels containing 200 ml of filtered seawater, [^{3}H]inulin, and α-[U-^{14}C]MG. The incubation vessels and tissue holders were maintained on a gyrotary shaker at 125 rpm. The system was designed so that as few as three tissues, or all the tissues (50) could be removed simultaneously and weighed wet, freeze-dried, or transferred to individual digestion tubes containing 1 *N* sodium hydroxide (NaOH). Samples of seawater were assayed for radioactivity at the beginning and end of each experiment to monitor constancy of the substrate concentration. Tissues were digested in 1 *N* NaOH at 50°C for 2–4 h, cooled, the volume adjusted, and then a sample counted in PCS (Amersham Corporation) scintillation fluor. A second sample was analyzed for total protein (Lowry *et al.*, 1951). Standard dual-channel counting procedures were used to assay for ^{3}H and ^{14}C. The accumulation of α-MG in the tissue was corrected for the inulin (extracellular) space (Schultz *et al.*, 1966).

Using these procedures, tissues were incubated for different lengths of time at the same substrate concentration or for fixed lengths of time at different substrate concentrations.

Transport product of α-MG

Tissues were incubated in α-[U-^{14}C]MG for up to 30 min. When removed, the tissues were quickly rinsed, frozen on an aluminum sheet (−70°C), and freeze-dried. The dry tissue was weighed, pulverized after cooling with liquid N_2, and homogenized in cold (4°C) water. The cold homogenizing tube was plunged into a boiling water bath and the contents heated rapidly to 91°C. The tube was removed, cooled slowly, and centrifuged at 16,000 × *g*. The supernatant was freeze-dried. For thin layer chromatography, the freeze-dried supernatant was dissolved in a small volume of water. A 20 μl sample was spotted on a silica-gel thin layer plate and developed in chloroform:methanol:water (60:70:26) (Kaback, 1968). The developed plates were scanned with a Varian Aerograph Radiochromatogram Scanner.

Effect of inhibitors and naphthalene on α-MG uptake

Inhibitors of Na^+K^+ transport (ouabain), glycolysis (iodoacetate), protonophores (DNP, CCCP, PCP), phloridzin (phloretin-2′-β-D-glucoside), and D-glucose were tested for their effect on α-MG uptake. Naphthalene, a compound known to hyperpolarize muscle cells (Nelson and Mangel, 1979), and stimulate glucose metabolism (Riley and Mix, 1981), was also tested. Ouabain, indoacetate, DNP, CCCP, PCP and naphthalene were dissolved in 80% ethyl alcohol before being diluted in the filtered seawater. Phloridzin and D-glucose were dissolved directly into the seawater. Fifty μl of ethyl alcohol was added to all treatments and controls.

The isolated gill tissues were pre-incubated for 30 min in 200 ml of filtered seawater containing either one of the inhibitors or naphthalene; and then transferred to a fresh set of incubation vessels containing 200 ml of filtered seawater, inhibitor or naphthalene, and 50 μM α-[U-^{14}C]MG. Tissues were then incubated for an additional 30 min. The incubation vessels were maintained on a gyrotory shaker at 125 rpm.

Leakage of α-MG

Tissues were incubated as described in the section on uptake of α-MG. However, after loading with α-[U-^{14}C]MG, 30–45 min, the tissues were transferred to tubes containing 4–8 ml of filtered seawater containing either no additives, 0.5 m*M* DNP, 0.25 m*M* CCCP, 0.25 m*M* PCP, 0.20 m*M* phloridzin or 0.20 m*M* D-glucose. The tubes were mixed (125 rpm) for 30 min. The tissues were then digested and a sample of the seawater counted as before. The effect of naphthalene on leakage was also determined. The seawater media used for loading tissues with α-[U-^{14}C]MG was made 1 μg ml^{-1} with naphthalene and then the tissues treated as described above.

The oysters used in this study were small. The wet weight of the gills was 0.150 g ± 0.076 (mean ($\bar{X}$) ± standard deviation (SD), n = 149). The inulin space was 0.415 ml (g wet wt)$^{-1}$ ± 0.102 ($\bar{X}$ ± SD, n = 80). This value compared reasonably well with the volume of the extracellular space in *Mytilus californianus* (46% of the whole body) (Thompson *et al.*, 1978).

RESULTS

Metabolism of α-MG

Gill tissue did not readily metabolize α-MG. Fifteen times as much glucose-carbon was converted to CO_2 as α-MG-carbon. After 1 h, 17.69% (SD = ± 6.09, n = 4) and 15.96% (SD = ± 2.88, n = 4) of the radioactivity from D-glucose and α-MG, respectively, were in the tissue digests and CO_2 traps. The remaining radioactivity was in the seawater. The percent of the accumulated dose (digests + CO_2) disposed to CO_2 was 14.49% (SD = ± 4.98, n = 4) for glucose and 0.97% (SD = ± 0.44, n = 4) for α-MG. The α-MG-carbon metabolized to CO_2 could partially be attributed to residual glucose contamination as a result of the synthesis of α-MG from glucose. α-MG was determined by the manufacturer to be 99% pure.

Uptake of α-MG

The α-MG was more concentrated in tissue than in the seawater. Uptake was linear over 90 min (Fig. 1a). The rate of α-MG uptake followed typical saturation kinetics, and adding D-glucose (50 μM) reduced the rate of uptake in a manner indicating competitive inhibition (Fig. 1b). Without glucose present the apparent K_t and V_{max} were estimated as 50.7 μM and 0.4216 μmol g-protein$^{-1} \cdot$ min^{-1}, respectively (Fig. 1c). In the presence of 50 μM glucose, the K_t and V_{max} were 148.5 μM and 0.4700 μmol g-protein$^{-1} \cdot$ min^{-1}, respectively. Glucose (50 μM) increased the apparent K_t by a factor of 3, but had little effect on V_{max}, thus indicating that α-MG and D-glucose uptake were mediated by the same transport system.

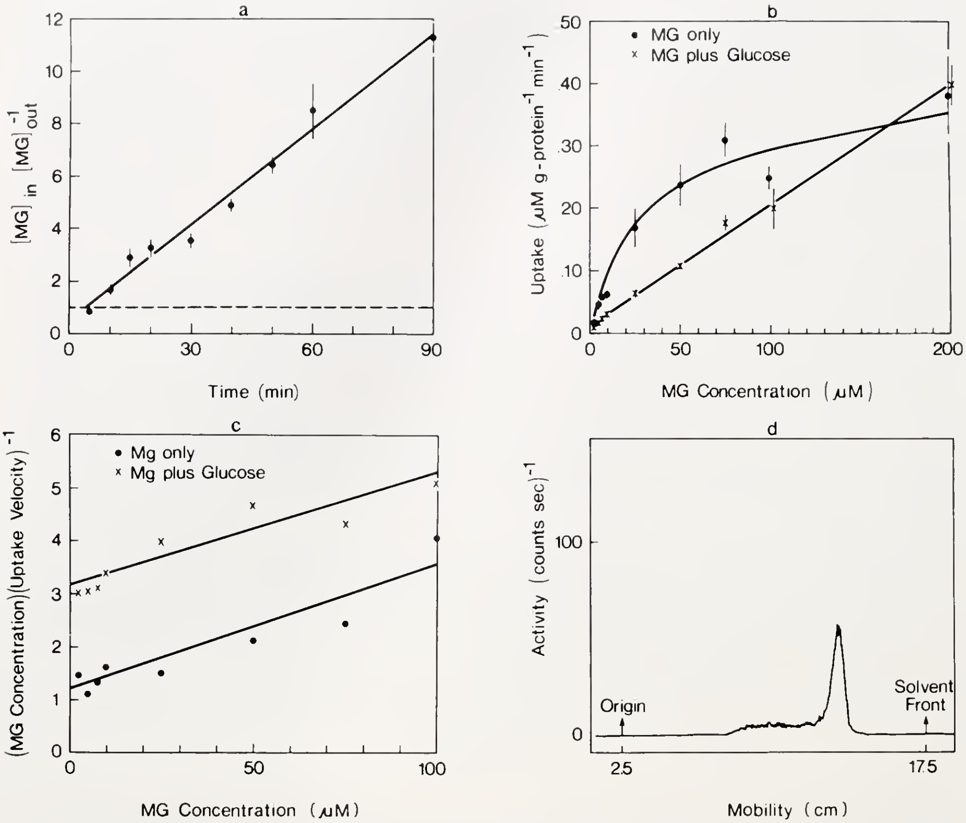

FIGURE 1. (a) The time course of α-MG uptake plotted as the ratio of α-MG in the tissue ($[\mathrm{MG}]_{\mathrm{in}}$) and α-MG in the seawater ($[\mathrm{MG}]_{\mathrm{out}}$). The concentration in the seawater was 20 μM. Each datum is the $\bar{\mathrm{X}} \pm$ SE, n = 4. The dotted line represents the α-MG uptake expected by diffusion alone. (b) The rate of α-MG uptake as a function of increasing substrate concentration with and without 50 μM D-glucose added. Each datum is the $\bar{\mathrm{X}} \pm$ SE, n = 3. (c) Linear transformation, Hanes-Woolf plot (Segel, 1976), of the data from (b). Slopes represent $1/\mathrm{V}_{\mathrm{max}}$ (entry max) and intercepts $\mathrm{K}_t/\mathrm{V}_{\mathrm{max}}$. (d) Typical radiochromatogram scan of the aqueous extract from α-Mg loaded tissues. The R_f of the single radioactive peak is the same as authentic α-MG (≃0.66).

Transport product of α-MG

Radiochromatogram scans indicated that all accumulated radioactivity was recovered as a single, well resolved peak (Fig. 1d) whose mobility was the same as the original α-MG prior to accumulation.

Effect of inhibitors and naphthalene on α-MG accumulation

Protonophores were the only metabolic inhibitors that significantly ($P < 0.05$) inhibited α-MG uptake (Fig. 2, 3). Concentrations of CCCP and PCP as low as 2.5 μM reduced uptake by 50%. However, even in the presence of 250 μM CCCP or PCP, uptake was greater than would be expected from diffusion alone. For diffusion alone, $[\mathrm{MG}]_{\mathrm{in}}\ [\mathrm{MG}]_{\mathrm{out}}^{-1}$ should equal 1.0.

Phloridzin was the only inhibitor that reduced α-MG uptake to less than ex-

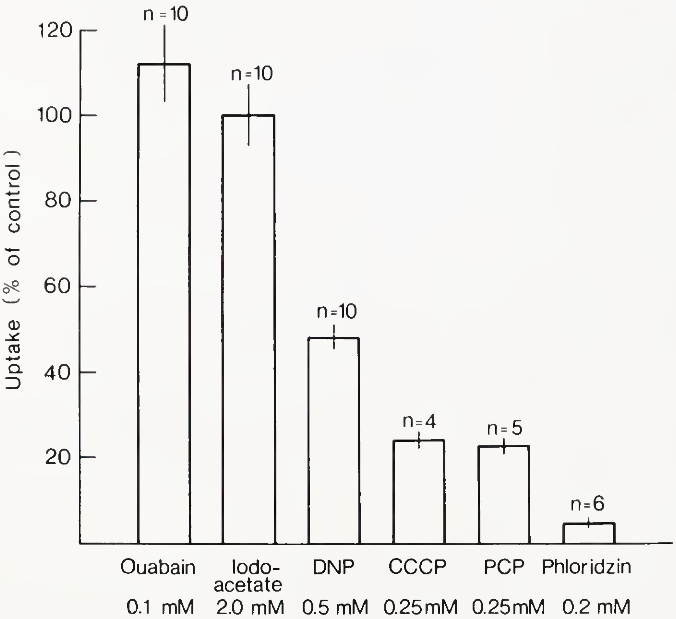

FIGURE 2. The effect of metabolic inhibitors and the competitive inhibitor, phloridzin, on α-MG uptake. The total incubation time with the inhibitor was 60 min, 30 min of which was with 50 μM α-MG. Values expressed as a percent (±SE) of the mean concentration ratio for controls, uncorrected for diffusion.

pected based on diffusion alone. The calculated uptake attributable to diffusion was 13.6% ± 2.9 ($\bar{X} \pm$ SD, n = 5) of the total α-MG uptake. Uptake in the presence of 200 μM phloridzin was 4.5% ± 0.6 ($\bar{X} \pm$ SD, n = 6) of the uptake of controls (Fig. 2); significantly less than expected based on diffusion alone.

Naphthalene-treated gills took up significantly greater amounts of α-MG than controls (Table I). Uptake of α-MG ranged from 132% to 141% of controls.

Leakage of α-MG

All inhibitors of α-MG uptake significantly ($P < 0.05$) increased leakage of ^{14}C-label from α-MG-loaded gills (Fig. 4). Phloridzin was least effective and D-glucose the most effective at stimulating leakage.

Naphthalene treatment slightly decreased leakage, but the effect was not significant ($P > 0.05$). Leakage of α-MG after 30 min was 1.26% ± 0.55 (n = 10) and 1.66% ± 0.63 (n = 9) for naphthalene-treated and control-treated gills, respectively.

DISCUSSION

D-glucose and α-MG were transported by the same carrier in the gill of *O. edulis*. Once accumulated, D-glucose was extensively metabolized to CO_2, while α-MG was not. Thus, α-MG is a transport analog of D-glucose not metabolized via the classical aerobic pathway of glucose metabolism. It was taken up against its chemical potential gradient, and preliminary evidence (one-dimensional TLC)

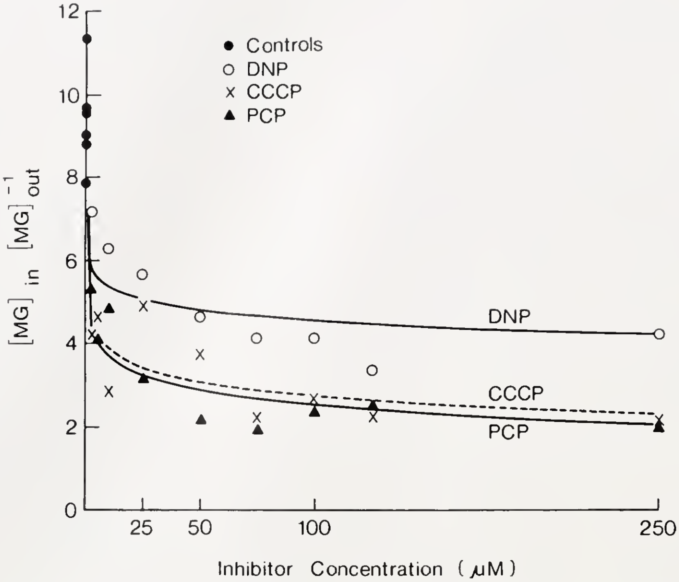

FIGURE 3. The effect of protonophore (DNP, CCCP, PCP) concentration on α-MG uptake. The total incubation time with the inhibitor was 60 min, 30 min of which was with 50 μM α-MG. The ratio of α-MG in the tissue ($[MG]_{in}$) to that in the seawater ($[MG]_{out}$) for the controls was 9.34 ± 0.48 ($\bar{X}$ ± SE, n = 6).

indicated that it accumulated chemically unaltered. In mammalian tissues, only the epithelial cells of the small intestine and kidney proximal tubules take up sugars against their chemical potential gradient (Crane, 1977), with the substrate specificity varying between tissues. The structural requirements of the oyster gill are similar to the rabbit renal brush border (Aronson and Sacktor, 1975). The glucose carrier in the oyster gill is specific for the hydroxyl group at the C_3 position on the glucose molecule, but not for the hydroxyl at C_1. The orientation of the hydroxyl at C_4 is not critical. These structural requirements were deduced from the fact that

TABLE I

Effect of 1 μg ml⁻¹ (initial concentration) naphthalene on α-MG uptake by isolated gills in three separate experiments (1 to 3). Values calculated as $[MG]_{in}$ $[MG]_{out}^{-1}$ ± 95% C. I.; number of tissues in parentheses. Ct: control-treated; Nt: Naphthalene-treated. The total incubation time with naphthalene was 60 min, 30 min of which was with 50 μM α-MG.

Experiment	Ct	Nt
1*	4.99 ± 0.83 (9)	6.56 ± 1.06 (9)
2**	5.36 ± 0.48 (10)	7.53 ± 0.82 (10)
3**	4.93 ± 0.53 (10)	6.96 ± 0.70 (10)

* $P < 0.025$; Student's *t* test.
**$P < 0.001$; Student's *t* test.

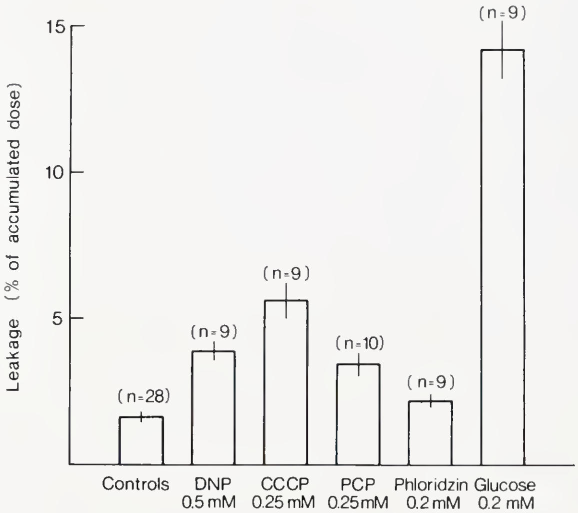

FIGURE 4. The effect of protonophores (DNP, CCCP, PCP) and competitive inhibitors (phloridzin, D-glucose) on α-MG leakage. Values expressed as a percent (±SE) of the total α-MG accumulated in the tissue after 30 min incubation in α-MG followed by 30 min leakage in the presence of the inhibitor.

D-glucose and D-galactose, but not 3-MG were taken up by the gill of *C. gigas* (Bamford and Gingles, 1974), and α-MG was taken up by *O. edulis.*

Comparison of kinetic data from different studies is of questionable validity since the physiological state of the tissue greatly influences the kinetics of transport. Wright (1979) demonstrated that isolation of gill filaments from *Mytilus californianus* reduced activity of the lateral cilia and subsequently increased apparent Michaelis constants (K_t) for glycine transport. However, study of uptake using isolated tissues is valid for studying mechanisms of transport and possibly for determining maximum transport velocities.

The fact that α-MG uptake was not reduced in the presence of ouabain suggests that either Na^+ transport out of the intracellular space is dependent on a ouabain insensitive pump, or that the ouabain sensitive Na^+ pump is inaccessible to ouabain dissolved in the external seawater medium. Sodium-gradient-coupled sugar and amino acid transport is well documented in mammalian tissues (Crane, 1977) and the gill epithelia of bivalves (Bamford and Gingles, 1974; Wright and Stephens, 1977). Typically, the basic model of the Na^+ gradient hypothesis requires 2 components: a mobile Na^+ plus substrate carrier and an energized Na^+ pump (Crane, 1977). In mammalian small intestines, the Na^+-coupled transport of glucose depends on the transmembrane electrochemical potential gradient, maintained by the Na^+ pump, which is the sum of the transmembrane chemical potential gradient (concentration ratio of Na^+ across the cell membrane) and the electrical membrane potential (Crane, 1977). Compounds which inhibit the Na^+ pump or abolish the membrane potential will also inhibit glucose accumulation. Therefore, it was surprising that ouabain, a compound known to inhibit the Na^+ pump in the plasma membrane, had no inhibitory effect on α-MG uptake by the oyster gill. Stewart and Bamford (1975) found that uptake of alanine by the gill of *Mya arenaria* was relatively insensitive to ouabain (2mM) unless tissue strips were first pre-incubated for 60 min. Since the gill-mantle complex of estuarine clams is known to be rich in Na^+K^+ ATPase activity (Saintsing and Towle, 1978), insensitivity to ouabain suggests that the Na^+ pump is inaccessible to ouabain dissolved in seawater. Silva *et al.* (1977) demonstrated in the American eel, *Anquilla rostrata*, that Na^+K^+ ATPase in gill epithelia pumps Na^+ into the extracellular fluid rather than external seawater. Ouabain circulating in the blood of the eel effectively inhibited Na^+K^+ ATPase in the gills while ouabain in seawater was much less effective (Silva *et al.*, 1977). Parallel considerations may also apply to oysters.

Compounds capable of collapsing the transmembrane electrical potential, such as DNP, CCCP, and PCP (Smejtek *et al.*, 1976) all reduced α-MG uptake by the oyster gill. This is consistent with the gradient hypothesis. In rabbit renal brush border membrane vesicles, the Na^+ dependent transport of D-glucose is an electrogenic process and thus directly related to the transmembrane electrical-potential gradient (Beck and Sacktor, 1975). Alternatively, inhibition of α-MG accumulation by protonophores could result from inhibition of ciliary activity due to depolarization of the membrane potential (Murakami and Takahashi, 1975), and this in turn could increase the unstirred layer around the gill cells and reduce α-MG uptake.

Glucose transport by the oyster gill may depend on the transmembrane electrical potential as well as the Na^+ gradient. Compounds known to depolarize cells inhibited α-MG uptake. Naphthalene, a compound shown to hyperpolarize muscle cells (Nelson and Mangel, 1979), stimulated α-MG uptake.

The cellular metabolic rate and the rate of glucose uptake are related in many mammalian tissues (Elbrink and Bihler, 1975). This may also be true for the oyster gill when the external glucose concentration is high. In intact oysters, the rate of glucose uptake may limit the rate of irreversible disposal of glucose (net outward transport from the endogenous glucose pool) if dissolved glucose is present in the external seawater (Riley, 1981). Thus, stimulating the glucose transport system should stimulate glucose metabolism. Naphthalene stimulation of glucose metabolism has been demonstrated (Riley and Mix, 1981).

Of all the inhibitors tested, only phloridzin reduced uptake to less than that expected by diffusion alone. Alvarado (1967) demonstrated that phloridzin was a non-penetrating inhibitor which binds so as to immobilize the carrier. In the oyster gill, immobilizing the carrier reduced accumulation in both intracellular and extracellular space, thus suggesting that entry into extracellular space must be preceded by passage through intracellular space via the mobile carrier.

The conclusion that the mobile carrier is the major pathway for entry and exit of α-MG is further substantiated by the fact that in the presence of 200 μM glucose, the total leakage was 6 times greater than in the presence of 200 μM phloridzin. Leakage in the presence of 200 μM glucose represents the sum of both inner and outer leaks, as defined by Crane (1977). Leakage in the presence of 200 μM phloridzin results from only outer leaks. Inner leaks are mediated by the mobile carrier; outer leaks are not. The difference between the two treatments is a relative measure of the importance of leakage via the mobile carrier. Inner leaks are clearly much more important than outer leaks in the oyster gill.

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LITERATURE CITED

ALVARADO, F. 1967. Hypothesis for the interaction of phloridzin and phloretin with membrane carriers for sugars. *Biochim. Biophys. Acta* **135:** 483–495.

ARONSON, P. S., AND B. SACKTOR. 1975. The Na^+ gradient-dependent transport of D-glucose in renal brush border membranes. *J. Biol. Chem.* **250:** 6032–6039.

BAMFORD, D. R., AND R. GINGLES. 1974. Absorption of sugars in the gill of the Japanese oyster, *Crassostrea gigas. Comp. Biochem. Physiol.* **49A:** 637–646.

BECK, J. C., AND B. SACKTOR. 1975. Energetics of the Na^+-dependent transport of D-glucose in renal brush border membrane vesicles. *J. Biol. Chem.* **250:** 8674–8680.

CRANE, R. K. 1977. The gradient hypothesis and other models of carrier mediated active transport. *Rev. Physiol. Biochem. Pharmacol.* **78:** 99–159.

ELBRINK, J., AND I. BIHLER. 1975. Membrane transport: The relation to cellular metabolic rates. *Science* **188:** 1177–1184.

KABACK, H. R. 1968. The role of the phosphoenol-pyruvate-phosphotransferase system in the transport of sugars by isolated membrane preparations of *Escherichia coli. J. Biol. Chem.* **243:** 3711–3724.

LOWRY, O. H., N. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

MARCHESI, V. T. 1979. Functional components of surface membranes: potential targets for pharmacological manipulation. *Pharmacol. Rev.* **30:** 371–381.

MURAKAMI, A., AND K. TAKAHASHI. 1975. Correlation of electrical and mechanical responses in nervous control of cilia. *Nature* **257:** 48–49.

NELSON, D. O., AND A. W. MANGEL. 1979. Naphthalene-induced crayfish muscle membrane hyperpolarization. *Comp. Biochem. Physiol.* **63C:** 309–311.

NETER, J., AND W. WASSERMAN. 1974. *Applied Linear Statistical Models.* Richard D. Irwin Inc., Homewood, Illinois.

PÉQUIGNAT, E. 1973. A kinetic and auto-radiographic study of the direct assimilation of amino acids and glucose by the organs of the mussel *Mytilus edulis. Mar. Biol.* **19:** 227–244.

PRITCHARD, J. B. 1979. Toxic substances and cell membrane function. *Fed. Proc.* **38:** 2220–2225.

RILEY, R. T. 1981. Aerobic glucose disposal in the oyster: An *in vivo* kinetic analysis. *Comp. Biochem. Physiol.* **68A:** 253–260.

RILEY, R. T., AND M. C. MIX. 1981. The effects of naphthalene on glucose metabolism in the European flat oyster *Ostrea edulis. Comp. Biochem. Physiol.* **70C:** 13–20.

SEGEL, I. H. 1976. *Biochemical Calculations, 2nd Ed.* John Wiley & Sons. New York.

SAINTSING, D. G., AND D. W. TOWLE. 1978. $Na^+ + K^+$ - ATPase in the osmoregulating clam *Rangia cuneata J. Exp. Zool.* **206:** 435–442.

SCHULTZ, S. G., R. E. FUISZ, AND P. F. CURRAN. 1966. Amino acid and sugar transport in rabbit ileum. *J. Gen. Physiol.* **49:** 849–866.

SILVA, P., R. SOLOMON, K. SPOKES, AND F. H. EPSTEIN. 1977. Ouabain inhibition of gill Na-K-ATPase: relationship to active chloride transport. *J. Exp. Zool.* **199:** 419–426.

SMEJTEK, P., K. HSU, AND W. PERMAM. 1976. Electrical conductivity in lipid bilayer membranes induced by pentachlorophenol. *Biophys. J.* **16:** 319–336.

STEWART, M. G., AND D. R. BAMFORD. 1975. Kinetics of alanine uptake by the gills of the soft shelled clam *Mya arenaria. Comp. Biochem. Physiol.* **52A:** 67–74.

THOMPSON, R. J., C. J. BAYNE, M. N. MOORE, AND T. H. CAREFOOT. 1976. Haemolymph volume, changes in the biochemical composition of the blood, and cytological responses of the digestive cells in *Mytilus californianus* Conrad, induced by nutritional, thermal and exposure stress. *J. Comp. Physiol.* **127:** 287–298.

VACCARO, R. F., AND H. W. JANNASCH. 1966. Studies on heterotrophic activity in seawater based on glucose assimilation. *Limnol. Oceanogr.* **11:** 596–607.

WEINER, M. W. 1979. Uncoupling agents distinguish between the effects of metabolic inhibitors and transport inhibitors. *Science* **204:** 187–188.

WRIGHT, S. H. 1979. Effect of activity of lateral cilia on transport of amino acids in gills of *Mytilus californianus. J. Exp. Zool.* **209:** 209–220.

WRIGHT, S. H., AND G. C. STEPHENS. 1977. Characteristics of influx and net flux of amino acids in *Mytilus californianus. Biol. Bull.* **152:** 295–310.

Reference: *Biol. Bull.* **161**: 426–439. (December, 1981)

SECRETION OF NITROGEN INTO THE SWIMBLADDER OF FISH. I. SECRETION BY FISHES NEARLY LACKING CIRCULATING HEMOGLOBIN. ROLE OF THE RETE MIRABILE[1]

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ABSTRACT

Toadfish (*Opsanus tau*) essentially lacking circulating erythrocytes were prepared by repeated exchange transfusion with serum. The rate of nitrogen secretion is not changed by removal of the erythrocytes. Oxygen secretion is slowed drastically. This shows that nitrogen secretion does not require erythrocytes and is not driven by oxygen secretion. In the absence of circulating erythrocytes, oxygen and nitrogen are brought into the swimbladder in proportion to their concentrations in blood plasma. Carbon dioxide partial pressure in the secreted gas mixture is three to fourfold greater than the pressure generated by acidifying arterial blood. This implies counter-current multiplication of the small increment of carbon dioxide pressure brought about by acidification of the blood. In the presence of blood buffers, increased carbon dioxide pressure will increase blood bicarbonate. Three independent estimates indicate that, during gas secretion, gas gland blood is near pH 6.5. Total carbon dioxide (CO_2, HCO_3^-, $CO_3^=$) is increased from the arterial value near 2 m*M* to about 14 m*M*, divided nearly equally between carbon dioxide and bicarbonate anion. The increment in total blood carbon dioxide concentration together with the well-known increment in lactate anion may serve to salt out inert gases from solution in blood plasma.

INTRODUCTION

The swimbladder of marine and fresh water teleost fishes is a gas filled sac which serves primarily to make the fish neutrally buoyant. The largest part of the gas brought into the swimbladder of most fish is oxygen, at a pressure very close to the external hydrostatic pressure. The difference in gas partial pressure in the contents of the swimbladder and in the lake or sea water is large in fishes living at any considerable depth, for the hydrostatic pressure increases about 1 atm with each 10 m depth; while the partial pressures of gases dissolved in lake or sea water are relatively independent of depth (Biot, 1807) and remain near 0.2 atm oxygen and 0.8 atm nitrogen. The partial pressure of oxygen in the swimbladders of marine fish living at 3000–6000 m depth is commonly 300 to 500 atm (Wittenberg *et al.*, 1980). A fish with a functional swimbladder has been captured from a depth of 7160 m (Nielsen and Munk, 1964); if the contained gas were 90% oxygen the partial pressure of oxygen would have been 644 atm.

Nitrogen and other chemically inert gases are brought into the swimbladder at partial pressures that greatly exceed their partial pressure in the surrounding

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[1] Dedicated to Professor David Shemin in honor of his 70th birthday.

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water. J. S. Haldane (1898), considering the analyses of swimbladder gas made by Biot (1807) 90 years earlier, realized that although nitrogen and argon make up only about 10% of the swimbladder gases, their partial pressure may be very great at the enormous pressures at which fish live in the deep sea. Haldane's estimate of the partial pressure of nitrogen in the swimbladders of deep sea fish has been extended from 7–8 atm to as high as 50 atm (Delaroche, 1809; Richard, 1895; Schloesing and Richard, 1896; Scholander *et al.*, 1951; Scholander and van Dam, 1953; Scholander, 1954; Kanwisher and Ebeling, 1957; Nielsen and Munk, 1964; Douglas, 1967; Wittenberg *et al.*, 1980).

Hüfner (1892) reported that the swimbladder gases of whitefish (*Coregonus acronius*) captured from the bottom of the Bodensee at a depth of 60 to 80 m contained 99% nitrogen. At the depth at which the fish were captured, the partial pressure of nitrogen in the swimbladder would have been 6–8 atm. Subsequently, nearly pure nitrogen at 10 atm pressure has been found in the swimbladders of many deep-living fresh and saltwater salmonids, coregonids, osmerids, and catostomids (Saunders, 1953; Scholander *et al.*, 1956a; Tait, 1956; Sundnes *et al.*, 1958; Sundnes, 1963; Sundnes *et al.*, 1969; Fahlén, 1970).

In Hüfner's (1892) classic study, the fraction of nitrogen in the swimbladder gas of most fish was 99%. However, substantial concentrations of oxygen (10–50% of the total gases) were found in some individual fish examined by Hüfner and by most later workers (Saunders, 1953; Tait, 1956; Fänge, 1958; Sundnes *et al.*, 1958; Fahlén, 1968; Fahlén, 1970). Sundnes (1963) finds that *Salmo* and *Coregonus*, which have 99% nitrogen in the swimbladder while residing for some weeks at one depth, may have as much as 70% oxygen while moving to deeper water during their annual spawning migration. Fahlén (1967b) emptied the swimbladder of *Coregonus* experimentally and found that the gases were replaced with a gas mixture containing up to 61% oxygen, and 6.5% carbon dioxide.

The swimbladder of fresh water cyprinids (goldfish, carp, roach, tench) is normally filled with pure nitrogen (Wittenberg, 1958). In fact, Priestley (quoted in de Fourcroy, 1789) used nitrogen from the swimbladder of carp purchased in the market for his chemical experiments. Nevertheless, if the swimbladders of these fish (Moreau, 1876, 1877; Krohn and Piiper, 1962; Evans and Damant, 1928; Wittenberg, 1958) are emptied experimentally, the swimbladder is refilled with a gas mixture containing up to 60% oxygen, and 2–10% carbon dioxide. Oxygen is removed from the swimbladder rapidly (Wittenberg, 1958; Piiper *et al.*, 1962), nitrogen slowly, thus nitrogen is accumulated in the swimbladders of those fishes that secrete gas slowly.

The fraction of nitrogen in the swimbladder gas of deep living marine fish tends to be constant for each species and independent of depth over a wide range (Scholander and van Dam, 1953; Scholander, 1954; Douglas, 1967). This implies that the rates of oxygen secretion and of nitrogen secretion remain in constant proportion for each species.

Oxygen secreted into the swimbladder comes from oxygen dissolved in the water in which the fish lives (Scholander *et al.*, 1956b; Wittenberg, 1961) and is brought to the swimbladder in the blood. Oxygen is released from the blood in the capillaries of the gas gland, a specialized area of the epithelium lining the swimbladder, and in the rete mirabile, a vascular counter-current exchange organ supplying blood to the gas gland (Denton, 1961; Steen, 1970). These structures are massive in fish that secrete oxygen rapidly and against high pressures (Marshall, 1960, 1972; Wittenberg, *et al.*, 1980). They are present, albeit inconspicuous and dispersed as small bundles of as few as two capillaries, in the salmonids, coregonids, and other fish that secrete gas slowly (Fänge, 1958; Marshall, 1960; Fahlén, 1967b, 1968, 1970, 1971). (Certain of these fishes, described as lacking retia mirabilia (Sundness, *et al.* 1958), in fact have retia (Fahlén, 1967b).) In these fish the rate of oxygen secretion may parallel the degree to which the microretia are developed (Fahlén, 1967b). Herring differ; they lack retia (Fahlén, 1967a; Blaxter *et al.*, 1979) and bring swallowed air into the swimbladder by way of the pneumatic duct.

We conclude that the gas brought into the swimbladder through the action of the gas gland and rete mirabile is always a mixture of nitrogen and oxygen.

In this study we have inquired whether, and how these two secretory processes are linked. We concluded that oxygen secretion and nitrogen secretion are independent processes driven by a common force. To prove this we have required fish lacking or nearly lacking circulating erythrocytes. In addition we have shown that the driving force for nitrogen secretion is generated by two separate processes—generation of lactate anion by aerobic glycolysis and counter-current multiplication of carbon dioxide pressure in the rete. An accompanying paper (Wittenberg *et al.*, 1981) discusses the molecular mechanism by which inert gases are driven from the blood plasma.

Materials and Methods

Toadfish

Toadfish (*Opsanus tau,* L.), weighing 250–600 g, were maintained, with feeding, in running sea water at 21°C. The morphology of the toadfish swimbladder has been described (Fänge and Wittenberg, 1958). Gases in the experimentally emptied swimbladder of fish maintained near atmospheric pressure are replaced in about 24 hours (Fänge and Wittenberg, 1958). All members of the population used in this study belong to the Type I hemoglobin phenotype described by Fyhn and Sullivan (1974) (86 individual fish were examined; all were type I; Sullivan, Bonaventura and Bonaventura, personal communication). This phenotype has six or more electrophoretically distinguishable hemoglobins.

Secretion of gas by toadfish breathing pure oxygen

A 100 liter tank was fabricated from plexiglass. The lid was made gas-tight with an O-ring seal. Temperature was maintained at 21°C by means of an external heat exchanger through which the water was circulated by a pump (TEEL model IP681, Dayton Electric, Chicago). A second pump circulated the water continuously through a bed of dolomitic limestone (Marine Filter Mix, Aquarium Systems Inc., Eastlake, Ohio), a small bed of activated charcoal and a layer of glass wool. A population of bacteria which developed in the filter bed after 5–6 days removed waste products, particularly the very toxic ammonia.

Abrupt exposure to high oxygen pressure injures toadfish. Accordingly, three to five 250-300 g fish were placed in the tank filled with air-equilibrated sea water, and the air replaced slowly by oxygen gas delivered from a bubbler at the bottom of the tank. The flow was more than 1 l per min. After 24 hours, the fish were restrained without anesthetic, and the swimbladder was emptied, using a syringe fitted with a fine needle. This initial gas sample was discarded. Thereafter gas samples were withdrawn for analysis at 24–48 h intervals. Three successive samples of secreted gas were often obtained from the same animal.

Gas analyses

Oxygen, carbon dioxide and residual inert gas were determined by the method of Scholander *et al.* (1955). Gas samples containing a large fraction of carbon dioxide were analyzed in the apparatus described by Fry *et al.* (1949) using the reagents of Scholander and Irving (1947).

Determination of blood hemoglobin

Washed red blood cells from a measured volume of blood were hemolyzed in distilled water; the solution was adjusted to pH 8.6 by the addition of sodium borate buffer, and the stroma removed by low speed centrifugation. The concentration of oxyhemoglobin was determined using a Beckman model DU spectrophotometer taking $\epsilon_{578\text{nm}} = 14.1 \cdot 10^3\ M^{-1}\ \text{cm}^{-1}$. Values are expressed as millimoles heme per liter blood (mM heme).

Saline and anticoagulant

A balanced salt solution for *Opsanus* was devised following the suggestions of Wolf and Quimby (1969) using the ionic composition of toadfish blood given Lahlou *et al.* (1969). It contained NaCl (170 mM), KCl (5.5 mM), $CaCl_2$ (1.5 mM), $MgCl_2$ (1.0 mM), $NaHCO_3$ (10 mM), KH_2PO_4 (0.6 mM). After aeration, the pH was 7.4. Heparin (500 IU/ml) was added to saline used to lubricate syringes or fill cannulae.

Serum

Blood was drawn from the heart or gill vessels. Red cells were removed by centrifugation and the hemoglobin-free plasma pooled and frozen. The clot discovered on thawing was removed by centrifugation at about 10,000 *g*.

Experimentally produced anemia

Procedures for prolonged cannulation of the dorsal aorta of free-swimming fish (Smith and Bell, 1964, 1967; Holeton and Randall, 1967) were adapted to the toadfish. Large toadfish (400–600 g) were anesthetized with Tricaine methanesulfonate (MS 22, Sandoz), and fine polyethylene tube (PE-10, Clay Adams, Inc.) filled with heparinized saline was implanted in the dorsal aorta at a point about 4 cm posterior to the vent. The PE-10 polyethylene tubing was connected to a one meter length of polyethylene tubing of larger bore (PE-50, Clay Adams, Inc.) filled with heparinized saline. Every 12 h blood equivalent to about 5% of the animal's weight was withdrawn from the unrestrained free-swimming fish using a syringe connected to the free end of the indwelling cannula. Since plasma proteins are replaced slowly in toadfish (Haschemeyer, 1973), this blood was replaced by an equal volume of toadfish serum.

Anemic toadfish showed no signs of distress while swimming freely, but were little able to withstand struggling or the stress of capture in a net. Accordingly, to obtain gas samples, Tricaine (400 mg per liter) was added to the sea water and the gas sample taken as soon as anesthesia was produced. Gas samples were taken every 48 hours.

Oxygen Equilibrium of Toadfish Blood

Blood from toadfish immobilized by intramuscular injection of d-tubocurarine (15 mg per kg) was drawn into syringes containing dry heparin, and was stirred for 20 minutes under oxygen to permit the consumption of any excess metabolites. Portions of blood were adjusted to known pH by the addition of small volumes of 0.2 *M* lactic acid in 0.2 *M* NaCl. The pH was verified at the end of each experiment and found unchanged. A drop of such blood was placed in a layer of 625-mesh woven stainless steel cloth, 0.1 mm thick, sandwiched between layers of teflon film (0.5 mil thick, Dilectrics Corporation, New York). Capillary flow brings the film of blood to a uniform thickness. This assembly was held in a pressure cuvette (Beckman RIIC, Ltd.) maintained at 20°C. Spectra were recorded using a Cary model 17 recording spectrophotometer equipped with a scattered transmission accessory. Calculations of fractional saturation of the hemoglobin with oxygen were made from the sum of optical density changes at 560 and 576 nm.

pH of toadfish blood equilibrated with oxygen and varying pressures of carbon dioxide

Portions of blood were equilibrated with 3 successive portions of each of the gas mixtures listed in Table I at 20°. The pH was determined with a Radiometer pH meter using a combined glass and reference electrode (Ingold Electrodes Incorporated, Lexington, Massachusetts).

Carbon dioxide pressure of acidified toadfish blood

Blood was withdrawn from the heart of toadfish immobilized by intramuscular injection of d-tubocurarine into syringes whose dead space was filled with a strong solution of heparin in 0.2 *M* NaCl. A portion of the blood sample was retained in the syringe and not exposed to air. A second portion ws equilibrated with air for 30 min at 20°C. Each portion was made first weakly, then strongly acidic by the addition of small volumes of lactic acid, 0.2 or 0.5 *M* in 0.2 *M* NaCl, and the pH and P_{CO_2} determined using a Radiometer E 5036-0 P_{CO_2} module and a PHM 72 Acid Base Analyser.

RESULTS

Toadfish nearly lacking blood hemoglobin

The blood hemoglobin concentration in normal toadfish of the population studied here was 2.35 ± 0.46 m*M* heme (mean and S.D. of 24 individuals, range 1.8–3.03).

TABLE I

pH of toadfish blood equilibrated with gas mixtures containing oxygen and carbon dioxide at 20°C.

	pH				
	CO_2, atmosphere				
Animal	0.0	0.040	0.088	0.135	0.189
520	7.67	7.03	6.74	6.60	6.50
521	8.34	7.25	7.00	6.84	6.71
522	7.89	7.00	6.66	6.54	6.40
523	8.04	6.95	6.64	6.47	6.36
524	7.93	6.99	6.68	6.49	6.38
525	7.80	6.96	6.66	6.47	6.36
mean ± SD	7.95 ± .23	7.03 ± .11	6.73 ± .14	6.57 ± .14	6.45 ± .14

The course of a series of exchange transfusions in which blood is withdrawn and replaced by serum is presented in Figure 1. A working lower limit of blood hemoglobin concentration is reached at 0.05 mM, about 2% of the normal value. Below this limit, removal of red blood cells appears to be countered by the mustering of new cells by the animal. The initial slope of the relation of Figure 1 suggests that about half of the blood volume is replaced at each exchange transfusion.

Composition of swimbladder gas in severely anemic fish

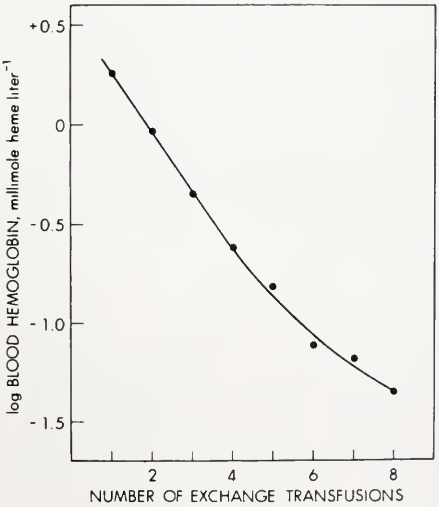
The fraction of oxygen in the gases brought into the swimbladder is much diminished as the level of blood hemoglobin is made less (Fig. 2). It does not, however, fall to zero but approaches about 35% in the most anemic fish examined. The ratio, $Ar/^{28}N_2$ (mean of the samples from the six most anemic fish in Fig. 2), is $1.40 \pm 0.10 \times 10^{-2}$.

It may be relevant to recall that the fraction of oxygen in gases secreted by toadfish whose blood hemoglobin is entirely combined with carbon monoxide also does not fall to zero (Wittenberg and Wittenberg, 1961).

Rate of gas secretion in severely anemic fish

The rate of replacement of oxygen in the experimentally emptied swimbladder falls precipitously to a low and more or less constant value as the level of blood hemoglobin is lowered.

The rate of inert gas secretion into the swimbladder of severely anemic fish is the same as the rate at which inert gas is brought into the swimbladders of normal



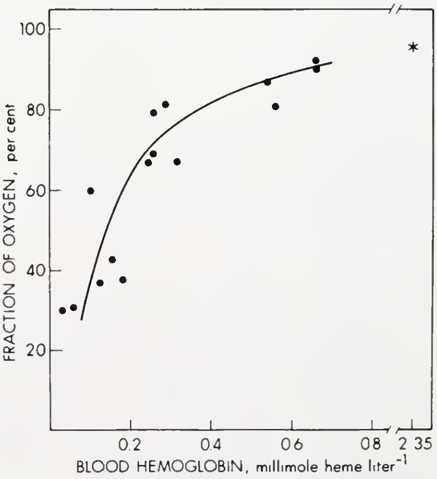


FIGURE 1. (Left) Blood hemoglobin concentration after successive exchange transfusions of an individual toadfish. Exchange perfusion was done every 12 hours.

FIGURE 2. (Right) Composition of the gas brought into the swimbladder as a function of blood hemoglobin concentration. The fraction of oxygen is expressed as a fraction of the sum of oxygen plus nitrogen, the fraction of carbon dioxide being variable. Dots represent individual gas samples; two successive gas samples were occasionally obtained from the same fish. The asterisk represents the mean of 50 samples of secreted gas and 24 blood samples from normal toadfish.

TABLE II

Rate of nitrogen secretion by toadfish, expressed as the fraction of the initial gas volume of the swimbladder replaced by inert gas (nitrogen plus argon) per 24 h.

Condition	Number of animals	Number of gas samples[b]	Rate of nitrogen secretion mean ± SD
Normal Fish; 1 atm	31	79	0.042 ± 0.035
Normal Fish; 6 atm	43	53	0.040 ± 0.035
Normal fish which had secreted gas at 6 atm and were subsequently maintained at 1 atm	20	20	0.052 ± 0.034
Severely anemic fish[a]	11	12	0.041 ± 0.034

a) Blood hemoglobin less than 0.29 mM. The normal hemoglobin level is 2.3 mM.

b) Often, three successive gas samples were obtained from each normal fish at 1 atm and two successive gas samples were obtained from normal fish at 6 atm.

fish at 1 atm or at 6 atm total pressure, (Table II). Details of the experiment in which fish were maintained at 6 atm pressure are presented elsewhere (Wittenberg *et al.*, 1981).

Oxygen equilibrium of toadfish blood

Oxygen equilibria were determined to a maximal P_{O_2} of 10 atm at 20°C using whole blood. In the relations presented in Figure 3, the solid symbols represent our data; the open symbols present data of the pioneers of this field, (Root, 1931; Green and Root, 1933; Root, *et al.*, 1939; Hall and McCutcheon, 1938). Their papers discuss the effects of protons and carbon dioxide. Our data serve to extend the range of oxygen pressure. These equilibria are complex; there are multiple inflections. Blood at arterial pH (pH = 7.65) is largely saturated with oxygen at any pressure greater than 0.12 atm oxygen. At $P_{O_2} = 5$ atm, the blood is largely saturated both at pH 7.65 and at pH 7.0; desaturation is noted at pH 6.5. At pH 6.5, the probable lower limit of acidity in the blood of the operating gas gland and rete mirabile, about 20% of the bound oxygen is driven from its combination with hemoglobin at 5 atm, and perhaps 60% at 1 atm P_{O_2}.

Carbon dioxide in gases brought into the swimbladder

Ordinarily the fraction of carbon dioxide in the gases brought into the toadfish swimbladder is low and variable, with occasional high values up to 18% of the total gas (Fänge and Wittenberg, 1958). This fraction became large and relatively constant in toadfish maintained in oxygen-equilibrated sea water. The average P_{CO_2} was about 0.10 atm, with occasional values to about 0.15 atm (Table III). Oxygen is 85–95% of the gas mixture.

pH and carbon dioxide pressure generated by acidifying toadfish blood

We wish to know whether the carbon dioxide pressure found in the swimbladder could have been generated by acidifying arterial toadfish blood. We assumed arterial P_{CO_2} is less than 2 torr, and equilibrated the blood with air. Steen (1963b)

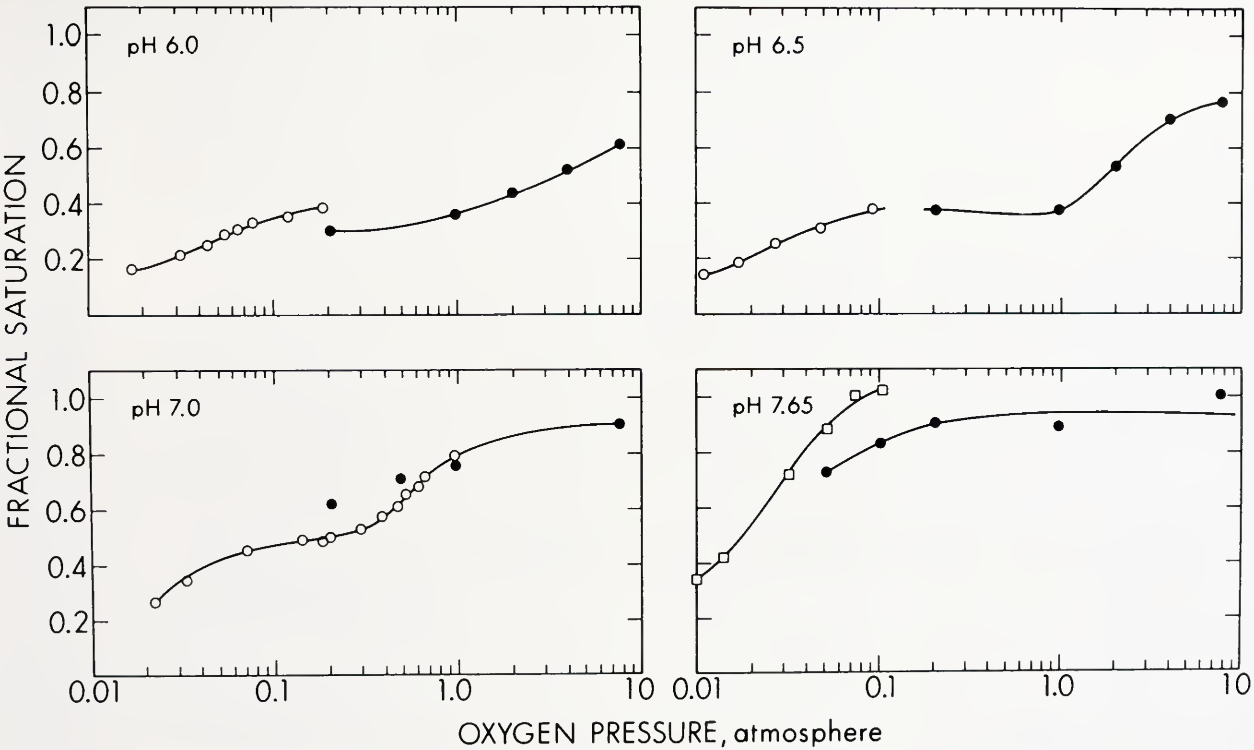


FIGURE 3. Oxygen equilibrium curves of toadfish whole blood. Open squares: data of Root (1931) obtained at 20°C and pH 7.60–7.99. Open circles: data of Green and Root (1933) obtained at 25°C. Solid circles: present data obtained at 20°C.

TABLE III

Composition of swimbladder gas of toadfish maintained in oxygen-equilibrated sea water. Samples marked A, B, C are successive samples from the same animal.

		Secreted Gas	
Animal	Interval (h)	Volume (% of initial gas volume)	CO_2 (%)
241	32	88	6.5
240A	24	100	6.5
B	32	79	7.1
503	24	50	8.5
504A	24	98	9.9
B	24	97	8.5
C	24	87	7.2
242	48	85	10.0
502A	24	81	11.2
B	24	85	7.5
C	24	81	11.2
239	48	63	12.0
509A	24	91	10.9
B	24	73	12.4
C	24	69	11.7
507	24	59	12.7
510	24	97	13.2
506	24	55	13.3
501A	24	81	14.5
B	24	83	11.6
C	24	71	10.7

records lactate levels of 13 ± 4 m*M* in the efferent gas gland vessel of the eel during gas secretion. Accordingly, we added lactic acid to about this concentration. Addition of lactic acid, 10 m*M*, to air equilibrated toadfish blood (Table IV) lowers the pH to about pH 6.5 and elevates the carbon dioxide partial pressure to 18 ± 5 torr, corresponding to 0.023 atm. Further addition of lactic acid to 25 m*M* brings the pH to the low value, pH 5.3 and increases the carbon dioxide pressure to 0.054 atm.

pH of blood in the gas gland capillaries

Three estimates may be made of the pH of blood in the gas gland capillaries of the toadfish. First, equilibration of oxygenated blood with carbon dioxide at the pressure obtaining in the swimbladder (0.10–0.12 atm) will bring the blood to about pH 6.5–6.7. The pH of toadfish blood as a function of P_{CO_2} is given in Table IV.

Second, addition of lactic acid to the concentration, 10 m*M* which probably obtains in the gas gland capillaries, will bring the blood to pH 6.5 (Table IV).

Third, toadfish maintained at 6 atm pressure continue to bring oxygen into the

TABLE IV

Carbon dioxide pressure generated by adding lactic acid to toadfish blood.

	Heart blood				Air equilibrated blood			
	10 m*M* Lactate		25m*M* Lactate		10 m*M* Lactate		25 m*M* Lactate	
Animal	pH	P_{CO_2} (torr)	pH	P_{CO_2} (torr)	pH	P_{CO_2} (torr)	pH	P_{CO_2} (torr)
748			5.40	62				
748			5.33	70				
749	6.44	32	5.45	77			5.50	28
750	6.46	30	5.41	77	6.65	11	5.40	33
751	6.30	39	5.11	90	6.51	16	5.14	43
752	6.32	35	5.07	89	6.48	22	5.05	59
753	6.45	44	4.81	87	6.58	22		

swimbladder, albeit at about half the normal rate (Wittenberg *et al.*, 1981). Inspection of the oxygen equilibrium of toadfish blood (Fig. 3) shows that at this pressure acidification to about pH 6.5 is required to liberate any large fraction of the bound oxygen. An estimated blood pH of 6.5 is consistent with all the data presented and is in good agreement with that found by Steen (1963b) by actual analysis of blood drawn from the efferent gas gland vessel of the eel.

DISCUSSION

To study the transport of the inert gases, nitrogen and argon, into the swimbladder of fish without interference from the simultaneous secretion of oxygen liberated from blood hemoglobin, we required fish lacking circulating erythrocytes. The famous icefish of the Antarctic lack erythrocytes (Ruud, 1954, 1965), but they do not have swimbladders, nor do they have the choroid rete mirabile (the oxygen-secreting, counter-current exchange organ of the eye; Wittenberg and Haedrich, 1974; Wittenberg and Wittenberg, 1974). Eels (Steen and Berg, 1966), toadfishes (Hall, 1929; Wittenberg and Wittenberg, 1961) and some frogs can survive without functional blood hemoglobin as long as they are not put under stress. In this study toadfish essentially lacking circulating erythrocytes are prepared by successive exchange transfusions. The blood hemoglobin concentration was reduced to about 0.05 m*M*, 2% of the already low normal value. This blood was a pale straw yellow detectably tinged with pink. For practical purposes, the most anemic fish we have prepared can be considered hemoglobin-free. The rate at which oxygen was replaced in the experimentally emptied swimbladder fell precipitously as the level of blood hemoglobin was lowered and reached a low and more or less constant value in the most anemic fish.

The rate at which nitrogen (and argon) were brought into the swimbladder is not affected by removal of the erythrocytes. Nor was this rate affected by increasing the hydrostatic pressure to 6 atmospheres, although the rate of oxygen secretion was slowed 2–4 fold. This, the central finding of our study, proves that nitrogen secretion into the swimbladder is not driven by oxygen secretion. Erythrocytes are not required for nitrogen secretion.

In the absence of erythrocyte hemoglobin, to which it may bind reversibly, oxygen behaves as any other gas. The gas mixture brought into the swimbladder of essentially erythrocyte-free fish contained oxygen and nitrogen approximately in the proportion 35:65. This is different from their proportion in air, 21:79, but is the same as their proportion, 35:65, in the gases dissolved in water which has been equilibrated with air. It follows that oxygen (in the absence of chemical binding) and nitrogen are brought into the swimbladder in proportion to their *concentrations* in the blood plasma. This implies salting out of nitrogen, oxygen and other gases from their solution in the plasma of the gas gland and rete capillaries (Wittenberg *et al.* 1981).

We enquire, what substances present in this plasma at enhanced concentration may be responsible for salting out the gases? In the eel which has a gas-secreting complex remarkably like that of the toadfish (Fänge and Wittenberg, 1958), the blood vessels are accessible and a partial answer is known. The cells of both the rete mirabile (Rasio, 1973) and gas gland (Kutchai, 1971) generate lactic acid by aerobic glycolysis of glucose brought in by the flowing blood. Arterial lactate in the eel ranges from about 2–10 m*M*. About 2.5 mmol lactic acid per l is added to the blood in its transit through the inflowing capillaries of the rete (bringing the blood to about pH 7.2). A further 5–6 mmol lactic acid per l is added in the capillaries of the gas gland, bringing the total lactic acid concentration to 13 ± 4 m*M* and bringing the blood to about pH 6.7 (Kuhn *et al.*, 1962; Steen, 1963b). We assume similar lactate levels in the gas gland capillaries of toadfish.

We learn the concentration of total carbon dioxide (CO_2, HCO_3^-, $CO_3^=$) in these vessels from the composition of the swimbladder gas. We assume only that the partial pressure of carbon dioxide in this blood approaches equilibrium with the gases of the swimbladder lumen, since the capillaries of the gas gland are separated from the gas-filled lumen by a single layer of cells about 30–50 μm thick. During refilling of the experimentally emptied swimbladder, particularly in toadfish maintained in oxygen equilibrated sea water, this carbon dioxide pressure is commonly 0.1 atm with occasional values to 0.15 or even 0.18 atm. This finding is not unusual; values from 0.1–0.37 atm have been reported for other species (Krohn and Piiper, 1962; Fänge, 1953; Scholander, 1956; Scholander *et al.*, 1956b; Wittenberg, 1961; Jacobs, 1930; Wittenberg *et al.*, 1964). The partial pressure of carbon dioxide in the gases brought into the swimbladder exceeds fivefold (bluefish) or three to fourfold (toadfish) the greatest pressure which could be generated by acidifying arterial blood (Wittenberg *et al.*, 1964). The source of this carbon dioxide is arterial blood; oxidative metabolism in the rete or gas gland contributes very little (Wittenberg *et al.*, 1964; Rasio, 1973). It follows that carbon dioxide pressure is assuredly augmented by counter-current multiplication in the rete mirabile (Kuhn *et al.*, 1963; Wittenberg *et al.*, 1964; Kuhn and Marti, 1966; Lesslauer *et al.*, 1966; Sund, 1977). The role of carbonic anhydrase, which is present in the rete at extraordinary concentration (reviewed in Wittenberg and Haedrich, 1974) remains unknown.

The total carbon dioxide concentration (CO_2, HCO_3^-, $CO_3^=$) in blood of the gas gland capillaries of the toadfish ($P_{CO_2} = 0.15$ atm; pH 6.5; 20°C) may be estimated from the carbon dioxide equilibrium curve of eel blood (Steen, 1963a), since blood buffering in eels (Steen, 1963a) and toadfish (Root, 1931) is about the same. It will be about 14 m*M*, divided about equally between CO_2 and HCO_3^-. This represents an increment of 12 m*M* total carbon dioxide above the arterial blood, which (at an estimated $P_{CO_2} = 2$ torr), will contain only 2 m*M* total carbon dioxide (Root, 1931).

Conclusion

From the foregoing facts we can deduce the sequence of events leading to the secretion of nitrogen against high pressure in the teleost swimbladder. We focus attention on blood plasma, since erythrocytes are not required for nitrogen secretion. The primary event must be the glycolytic generation of lactate anion and protons. Protons, reacting with blood bicarbonate, increase the partial pressure of carbon dioxide. This small "single concentrating effect" is cascaded by counter-current multiplication of the pressure of the highly diffusible carbon dioxide in the rete mirabile to give a much elevated partial pressure of carbon dioxide in the gas gland blood plasma. Interaction of carbon dioxide with blood buffers leads to a much increased concentration of total carbon dioxide (CO_2, $H_2CO_3^-$, $CO_3^=$) in the gas gland blood. This increment in the concentration of bicarbonate anion (and other forms of CO_2), in consort with the increment in the concentration of lactate anion, serves to salt out inert gases from their solution in the blood plasma.

Studies of natural populations and analyses of gases brought into the experimentally emptied swimbladder have shown that oxygen secretion and nitrogen secretion are closely linked processes. Protons, arising from dissociation of lactic acid, drive oxygen from its combination with blood hemoglobin, and thus provide the link between oxygen secretion and nitrogen secretion.

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LITERATURE CITED

Biot, J. B. 1807. Sur la nature de l'air contenue dans la vessie natatoire des poissons. *Mem. Phys. Chim. Soc. D'Arcuiel.* **1:** 252–281.

Blaxter, J. H. S., E. J. Denton, and J. A. B. Gray. 1979. The herring swimbladder as a gas reservoir for the acousticolateralis system. *J. Mar. Biol. Assoc. U. K.* **59:** 1–10.

de Fourcroy, A. F. 1789. Observations sur le gaz azote contenu dans la vessie natatoire de la carpe; et deux nouveaux procedes pour obtenir ce gaz. *Ann. Chim.* Paris, **1:** 47–51.

Delaroche, F. 1809. Observations sur la vessie aerienne des poissons. *Ann. Mus. Hist. Nat.* (Paris), **14:** 165–183.

Denton, E. J. 1961. The buoyancy of fish and cephalopods. *Prog. Biophys.* **11:** 177–234.

Douglas, E. L. 1967. Studies of gas secretion in the swimbladder of fishes. Ph.D. Dissertation, University of California, San Diego. Pp. 1–117 University Microfilms Inc., Ann Arbor, Michigan.

Evans, H. M., and G. C. C. Damant. 1928. Observations on the physiology of the swimbladder in cyprinoid fishes. *J. Exp. Biol.*, **6:** 42–55.

Fahlén, G. 1967a. Morphological aspects on the hydrostatic function of the gas bladder of *Clupea harengus. Acta Univ. Lund, Sect II*, No. 1, 1–49.

Fahlén, G. 1967b. Morphology of the gas bladder of *Coregonus lavaretus* L. *Acta Univ. Lund, Sect II*, No. 28: 1–37.

Fahlén, G. 1968. The gas bladder as a hydrostatic organ in *Thymallus thymallus*, L., *Osmerus eperlanus*, L., and *Mallotus villosus* Mull. *Fisk. Dir. Skr. Ser. HavUnnders.* **14:** 199–228.

Fahlén, G. 1970. The gas bladder of *Argentina silus*, L., with special reference to the ultrastructure of the gas gland cells and the counter-current vascular bundles. *Z. Zellforsch.* **110:** 350–372.

Fahlén, G. 1971. The functional morphology of the gas bladder of the genus Salmo. *Acta Anat.* **78:** 161–184.

Fänge, R. 1953. The mechanisms of gas transport in the swimbladder of euphysoclists. *Acta Physiol. Scand.* 30, Suppl. 110, Pp. 1–133.

Fänge, R. 1958. The structure and function of the gas bladder in *Argentina silus. Quart. J. Micr. Sci.* **99:** 95–102.

Fänge, R., and J. B. Wittenberg. 1958. The swimbladder of the toadfish (*Opsanus tau* L.) *Biol. Bull.* **115:** 172–179.

Fry, F. E. J., A. C. Burton, and O. G. Edholm. 1949. A simple gas analyzer. *Can. J. Res.*, Ser. E. **27:** 188–294.

Fyhn, U. E. H., and B. Sullivan. 1974. Hemoglobin polymorphism in fishes. I. Complex phenotypic patterns in the toadfish, *Opsanus tau. Biochem. Genetics* **11:** 373–385.

Green, A. A., and R. W. Root. 1933. The equilibrium between hemoglobin and oxygen in the blood of certain fishes. *Biol. Bull.* **64:** 383–404.

Haldane, J. S. 1898. Secretion and absorption of gas in the swimming-bladder and lungs. Part I. Swimming-bladder. *Sci. Prog.* **7:** 120–130.

Hall, F. G. 1929. The influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes. *Amer. J. Physiol.* **88:** 212–218.

Hall, F. G., and F. H. McCutcheon. 1938. The affinity of hemoglobin for oxygen in marine fishes. *J. Cell Comp. Physiol.* **11:** 205–212.

Haschemeyer, A. E. V. 1973. Kinetic analysis of synthesis and secretion of plasma proteins in a marine teleost. *J. Biol. Chem.* **248:** 1643–1649.

Holeton, G. F., and D. J. Randall. 1967. Changes in blood pressure in the rainbow trout during hypoxia. *J. Exp. Biol.* **46:** 297–305.

Hüfner, G. 1892. Zur physikalischen Chemie der Schwimmblasengase, *Arch. Anat. u. Physiol.* Physiol. Abt., 54–80.

Jacobs, W. 1930. Untersuchungen zur Physiologie der Schwimmblase der Fische. Uber die "Gassekretion" in der Schwimmblase von Physoklisten. *Z. vergleich. Physiol.* **11:** 565–629.

Kanwisher, J., and A. Ebeling. 1957. Composition of the swimbladder gas in bathypelagic fishes. *Deep Sea Res.* **4:** 211–217.

Krohn, H., and J. Piiper. 1962. Gassekretion in die Schwimmblase der Schleie (*tinca, tinca,* L.) in Wasser mit erniedrigtem N_2-Druck. *Naturwiss.* 48, 428–429.

Kuhn, H. J., and E. Marti. 1966. The active transport of oxygen and carbon dioxide into the swimbladder of fish. *J. Gen. Physiol.* **49:** 1209–1220.

Kuhn, H. J., P. Moser, and W. Kuhn. 1962. Haarnadelgegenstrom als Grundlage zur Erzeugung hoher Gasdrucke in der Schwimmblase von Tiefseefischen. *Plfuger's Arch. ges. Physiol.* **275:** 231–237.

Kuhn, W., A. Ramel, H. J. Kuhn, and E. Marti. 1963. The filling mechanism of the swimbladder. Generation of high gas pressures through hairpin countercurrent multiplication. *Experientia.* **19:** 497–511.

Kutchai, H. 1971. Role of carbonic anhydrase in lactate secretion by the swimbladder. *Comp. Biochem. Physiol.* **39A:** 357–359.

Lahlou, B., I. W. Henderson, and W. H. Sawer. 1969. Renal adaptation by *Opsanus tau*, a euryhaline aglomerular teleost to dilute media. *Am. J. Physiol.* **216:** 1266–1272.

Lesslauer, W., F. Burger, H. J. Kuhn, and W. Kuhn. 1966. Modellversuch zur Gaskonzentrierung in der Schwimmblase der Fische. *Pfluger's Archiv. fur ges. Physiol.* **290:** 56–60.

Marshall, N. B. 1960. Swimbladder structure of deep-sea fishes in relation to their systematics and biology. *Discovery Reports* **31:** 1–122.

Marshall, N. B. 1972. Swimbladder organization and depth ranges of deep-sea teleosts. In: *Symp. Soc. Exptl. Biol. XXVI, The Effects of Pressure on Organisms.* Pp. 261–272, Academic Press, New York.

Moreau, F. A. 1876. Recherches experimentales sur les fonctions de la vessie natatoire. *Ann. Sci. Nat. Zool.* ser. 6, **4:** 1–85.

Moreau, F. A. 1877. Memoires de Physiologie, Paris, G. Masson et Cie.

Nielsen, J. G., and O. Munk. 1964. A hadal fish (*Bassogigas profundissimus*) with a functional swimbladder. *Nature* **204:** 594–595.

Piiper, J., H. T. Humphrey, and H. Rahn. 1962. Gas composition of pressurized, perfused gas pockets and the fish swimbladder. *J. Appl. Physiol.* **17:** 275–282.

Rasio, E. A. 1973. Glucose metabolism in an isolated blood capillary preparation. *Canad. J. Biochem.* **51:** 701–708.

Richard, J. 1895. Sur les gaz de la vessie natatoire des poissons. *Compt. Rend. Acad. Sci.* (Paris), **120:** 745–747.

Root, R. W. 1931. The respiratory function of the blood of marine fishes. *Biol. Bull.* **61:** 427–456.

Root, R. W., L. Irving, and E. C. Black. 1939. The effect of hemolysis upon the combination with oxygen with the blood of some marine fishes. *J. Cell. Comp. Physiol.* **13:** 303–313.

Ruud, J. T. 1954. Vertebrates without erythrocytes or blood pigment. *Nature* **173:** 848–850.

Ruud, J. T. 1965. The ice fish. *Sci. Amer.* **213:** (November), 108–114.

Saunders, R. L. 1953. The swimbladder gas content of some freshwater fish with particular reference to the physostomes. *Can. J. Zool.* **31:** 547–560.

Schloesing, T., and J. Richard. 1896. Recherche de l'argon dans le gaz de la vessie natatoire des poissons et des Physalies. *Compt. Rend. Acad. Sci.* (Paris). **122:** 615–617.

Scholander, P. F. 1954. Secretion of gases against high pressures in the swimbladder of deep sea fishes. II. The rete mirabile. *Biol. Bull.* **107:** 260–277.

SCHOLANDER, P. F. 1956. Observations on the gas gland in living fish. *J. Cell. Comp. Physiol.* **48:** 523–528.

SCHOLANDER, P. F., C. L. CLAFF, C. T. TENG, AND V. WALTERS. 1951. Nitrogen tension in the swimbladder of marine fishes in relation to the depth. *Biol. Bull.* **101:** 178–193.

SCHOLANDER, P. F., AND C. IRVING. 1947. Micro blood gas analysis in fractions of a cubic millimeter of blood. *J. Biol. Chem.* **169:** 561–569.

SCHOLANDER, P. F., AND L. VAN DAM. 1953. Composition of the swimbladder gas in deep sea fishes. *Biol. Bull.* **104:** 75–86.

SCHOLANDER, P. F., L. VAN DAM, C. L. CLAFF, AND J. W. KANWISHER. 1955. Microgasometric determination of dissolved oxygen and nitrogen. *Biol. Bull.* **109:** 328–334.

SCHOLANDER, P. F., L. VAN DAM, AND T. ENNS. 1956a. Nitrogen secretion in the swimbladder of whitefish. *Science* **123:** 59–60.

SCHOLANDER, P. F., L. VAN DAM, AND T. ENNS. 1956b. The source of oxygen secreted into the swimbladder of cod. *J. Cell. Comp. Physiol.* **48:** 517–522.

SMITH, L. S., AND G. R. BELL. 1964. A technique for prolonged blood sampling in free-swimming salmon. *J. Fish. Res. Bd.* Canada **21:** 711–717.

SMITH, L. S., AND G. R. BELL. 1967. Anesthetic and surgical techniques for Pacific salmon. *J. Fish. Res. Bd.* Canada **24:** 1579–1588.

STEEN, J. B. 1963a. The physiology of the swimbladder of the eel, *Anguilla vulgaris.* I. The solubility of gases and the buffer capacity of the blood. *Acta Physiol. Scand.* **58:** 124–137.

STEEN, J. B. 1963b. The physiology of the swimbladder of the eel *Anguilla vulgaris.* III. The mechanism of gas secretion. *Acta Physiol. Scand.* **59:** 221–241.

STEEN, J. B. 1970. The swimbladder as a hydrostatic organ. In: W. S. Hoar and O. J. Randall, Eds., *Fish Physiology,* Vol. 4, Pp. 413–443, Academic Press, New York.

STEEN, J. B., AND T. BERG. 1966. The gills of two species of haemoglobin-free fishes compared to those of other teleosts with a note on severe anaemia in an eel. *Comp. Biochem. Physiol.* **18:** 517–526.

SUND, T. 1977. A mathematical model for counter-current multiplication in the swimbladder. *J. Physiol.* 267, 679–696.

SUNDNES, G. 1963. Studies on the high nitrogen content in the physostome swimbladder. *Fisk. Dir. Skr.* Ser. HavUnders **13:** 1–8.

SUNDNES, G., P. BRATLAND, AND E. STRAND. 1969. The gas content in the coregonid swimbladder. *Fisk. Dir. Skr.* Ser. HavUnders. **15:** 274–278.

SUNDNES, G., T. ENNS, AND P. F. SCHOLANDER. 1958. Gas secretion in fishes lacking rete mirabile. *J. Exp. Biol.* **35:** 671–676.

TAIT, J. S. 1956. Nitrogen and argon in salmonoid swimbladders. *Canad. J. Zool.* **34:** 58–62.

WITTENBERG, D. K., W. WITTENBERG, N. ITADA, AND J. B. WITTENBERG. 1981. Secretion of nitrogen into the swimbladder of fish. II. molecular mechanism. Secretion of noble gases. *Biol. Bull.* **161:** 440–451.

WITTENBERG, J. B. 1958. The secretion of inert gas into the swimbladder of fish. *J. Gen. Physiol.* **41:** 783–804.

WITTENBERG, J. B. 1961. The secretion of oxygen into the swimbladder of fish. I. The transport of molecular oxygen. *J. Gen. Physiol.* **44:** 521–526.

WITTENBERG, J. B., D. E. COPELAND, R. L. HAEDRICH, AND J. S. CHILD. 1980. The swimbladder of deep-sea fish: the swimbladder wall is a lipid-rich barrier to oxygen diffusion. *J. Mar. Biol. Assoc. U. K.* **60:** 263–276.

WITTENBERG, J. B., AND R. L. HAEDRICH. 1974. The choroid rete mirabile of the fish eye. II. Distribution and relation to the pseudobranch and to the swimbladder rete mirabile. *Biol. Bull.* **145:** 137–156.

WITTENBERG, J. B., M. J. SCHWEND, AND B. A. WITTENBERG. 1964. The secretion of oxygen into the swimbladder of fish. III. The role of carbon dioxide. *J. Gen. Physiol.* **48:** 337–355.

WITTENBERG, J. B., AND B. A. WITTENBERG. 1961. The secretion of oxygen into the swimbladder of fish. II. The simultaneous transport of carbon monoxide and oxygen. *J. Gen. Physiol.* **44:** 527–542.

WITTENBERG, J. B., AND B. A. WITTENBERG. 1974. The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: comparison with the swimbladder rete mirabile. *Biol. Bull.* **145:** 116–136.

WITTENBERG, J.B., W. WITTENBERG, AND D. K. WITTENBERG. 1976. Role of blood plasma and erythrocytes in secretion of inert gas into the teleost swimbladder. *Biol. Bull.* **151:** 434–435.

WOLF, K., AND M. C. QUIMBY. 1969. Fish cell and tissue culture. Pp. 253–305 In: W. S. Hoar and D. J. Randall, Eds. *Fish Physiology*, Vol. III, Academic Press, New York.

Reference: *Biol. Bull.* **161:** 440–451. (December, 1981)

SECRETION OF NITROGEN INTO THE SWIMBLADDER OF FISH. II. MOLECULAR MECHANISM. SECRETION OF NOBLE GASES[1]

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ABSTRACT

Toadfish (*Opsanus tau*) were maintained at 50 m depth, 6 atm total pressure. The partial pressures of argon and nitrogen in the gases brought into the experimentally emptied swimbladder exceed the ambient pressures. The fraction of nitrogen in the gases brought into the swimbladder is nearly independent of depth. This finding is inconsistent with an earlier hypothesis that active oxygen secretion, by forming minute bubbles, drives nitrogen secretion. Toadfish were maintained in seawater equilibrated with mixtures containing oxygen, nitrogen, helium (in previous experiments), neon, argon, krypton and xenon. The more soluble gases are enriched in the mixture brought into the swimbladder, so that the composition of the inert gases brought into the swimbladder is similar to the composition of the gases dissolved in blood plasma. The enhancements, ($[Gas/N_2]_{secreted} \div [Gas/N_2]_{ambient}$), of the gases in the mixture brought into the swimbladder are proportional to the solubility of the gases in water. These facts support the hypothesis that salting out of inert gases elevates the partial pressure of nitrogen and other inert gases in the gas gland blood vessels. High gas pressures may be generated by counter-current multiplication of this initial effect.

INTRODUCTION

Nitrogen and other chemically inert gases may be brought into the swimbladder of fishes in the face of very large pressures. Hüfner (1892) found nearly pure nitrogen under 5–7 atmospheres pressure in the swimbladders of whitefish captured from deep in the Bodensee. Schloesing and Richard (1896) found in the swimbladder of a deep sea eel captured from 1385 m, 14.0% nitrogen, 0.278% argon, 79.6% oxygen, and 6.1% carbon dioxide. J. S. Haldane (1898) pointed out that this implied active secretion of both argon and nitrogen. At the depth from which the animal was captured, the partial pressures of nitrogen and argon would have been 19.4 and 0.39 atm, twenty-fivefold greater than the corresponding pressures in sea water. While one might conceive of a chemical mechanism to capture and transport oxygen or even nitrogen, the noble gas, argon, forms no compounds, and the mechanism by which it was brought into the swimbladder could not involve formation of a chemical bond to a carrier molecule.

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The facts have been abundantly verified. The mass ratio, argon/nitrogen, in the gases present at the time of capture in the swimbladders of deep (Schloesing and Richard, 1896; Scholander, 1954; Douglas, 1967) and shallow-living (Wittenberg, 1958; Wittenberg *et al.*, 1964) marine fishes and of deep-living coregonids and salmonids (Scholander, *et al.*, 1956; Tait, 1956; Sundness *et al.*, 1958) is always close to that of air, 1.20×10^{-2}. In sharp contrast, the mass ratio, argon/nitrogen, in the gases as they enter the swimbladder may be far from that of air (Wittenberg, 1958). This fact is the starting point of the present study.

Two divergent theories have been offered to account for inert gas secretion. Koch (1934) suggested that inert gases might be expelled from the blood flowing through the rete mirabile if the gas solubilities were somehow depressed through the action of the gas gland. When it was discovered that lactic acid, generated by aerobic glycolysis, was present at high concentration in this blood, Kuhn and his colleagues (Kuhn and Kuhn, 1961; Kuhn *et al.*, 1963) and Scholander and his colleagues (Scholander, 1954; Enns *et al.*, 1967) calculated that salting out of gases with counter-current multiplication of the resulting "single concentrating effect" in the rete mirabile could account for the highest pressures of inert gases found in the swimbladder.

Powers (1932) suggested that gas bubble evolution might somehow be involved, and Wittenberg (1958) attempted to show that generation of oxygen bubbles could provide the driving force for inert gas secretion. The experiments to be presented here remove the experimental basis for the bubble hypothesis.

Here, we have examined the relative rates at which nitrogen and several of the noble gases are brought into the swimbladder. We found that the inert gases and dissolved oxygen were brought into the swimbladder in proportion to their concentrations in the blood plasma. This implies that they were driven from watery solution by some process that depressed their solubility. Salting out is one such process. Processes strongly influenced by relative rates of diffusion of the gases are ruled out.

In an accompanying paper (Wittenberg *et al.*, 1981) we showed that inert gas secretion persisted unchanged in the absence of circulating erythrocytes, and we estimated the increments of lactate anion, protons and carbon dioxide added to plasma of blood circulating through the gas-secreting complex.

MATERIALS AND METHODS

Gas analyses

Oxygen, carbon dioxide and residual inert gases were determined as previously (Wittenberg *et al.*, 1981).

The ratios of noble gases to nitrogen were determined using a mass spectrometer (model 21-620A of the Consolidated Electrodynamic Corporation). Oxygen was not removed prior to analysis. Air served as a standard for argon and $^{28}N_2$. Unfortunately, the instrument used could not be adapted to determine the ratio of helium to gases of much greater molecular weight. Filaments of earlier mass spectrometers were destroyed by oxygen, and in early experiments (Wittenberg, 1958) it was necessary to remove oxygen from the samples prior to analysis, with some consequent distortion of the mass ratios. This objection applies to the data for helium quoted here.

Secretion of gas by toadfish at six atmospheres pressure

Toadfish were maintained in live cars at the bottom of the deepest water available to us, a 50 m deep depression in the sea floor (Lat. 41° 27′ 2″ N; Long. 70° 57′ 9″ W) near Cuttyhunk Island, Massachusetts. The depth and temperature were determined with a bathythermograph, and the depth was corroborated with a calibrated depth sounder. The pressure at 50 m depth corresponds to 5 atm hydrostatic pressure from the overlying water column plus 1 atm atmospheric pressure, a total of 6 atm. The water at this location is stirred by strong tidal currents. The temperature at depth was 19.6°C, the surface temperature 21–22°C.

The swimbladder was emptied by means of a syringe fitted with a fine needle. The fish were lowered to depth. After 48 h the fish were brought to the surface; the swimbladder gases withdrawn immediately, and the fish returned to depth for a second 48 h period.

Secretion of gas by toadfish breathing a mixture of oxygen, nitrogen and noble gases

The apparatus was the same as that described (Wittenberg *et al.*, 1981) except that gases were recirculated continuously from a 100 l external reservoir. A bed of "Baralyme" (Thomas A. Edison Co., Stuyvesant Falls, New York) interposed between the tank and reservoir served to remove carbon dioxide. The recirculating gases contained argon-free oxygen and a mixture of inert gases. In one experiment this mixture contained: neon, 2.1%; argon 2.0%; krypton, 2.0%; nitrogen, 93.9%. In a separate experiment, the mixture contained argon, 1.9%; krypton, 2.0%, xenon, 2.0%, and nitrogen, 94.1%. At the beginning of each experiment seawater in the tank was equilibrated with three successive portions of the gas mixture; these portions were discarded and the reservoir filled. Oxygen in the gas mixture was replenished daily or more frequently with argon-free oxygen so as to maintain the fraction of oxygen in the gas mixture at 21%. Samples of the recirculating gas mixture were taken frequently for comparison with the gases brought into the swimbladder. Fish were introduced into the tank at least 24 h before the swimbladder was emptied initially. The initial gas sample was discarded and subsequently samples were taken for analysis every 12 or 24 hours. Frequently 3 or 4 successive samples were obtained from each fish.

Presentation of mass spectrographic data

The mass spectrometer reports the ratios of the number of ions of each apparent mass. We take nitrogen as a common point of reference and consider the ratios of the mass abundance of each inert gas to the abundance of mass 28, diatomic ^{14}N. To compare gases brought into the swimbladder with ambient levels we define a quantity, "enhancement" (relative to nitrogen), which is a ratio of the experimentally determined mass ratios:

$$\left(\frac{\text{noble gas}}{\text{mass 28}}\right)_{\text{secreted}} \div \left(\frac{\text{noble gas}}{\text{mass 28}}\right)_{\text{ambient}}$$

Here, ambient is taken as the gas phase with which the sea water is in equilibrium.

The mass ratio, noble gas/nitrogen, will be different for gases dissolved in water and for the gas phase with which the water phase is in equilibrium. For instance, the mole fractions of argon and nitrogen in air are 0.00934 and 0.7808, respectively.

Their mass ratio is $^{40}Ar/^{28}N_2 = 0.0120$. Argon, which is 2.19-fold more soluble in water than nitrogen (at 20°C), is enriched 2.19-fold in the gases dissolved in air-equilibrated water. Consequently the mass ratio in the dissolved gases is $^{40}Ar/^{28}N_2 = 2.19 \times 0.012 = 0.026$.

RESULTS

Secretion of gas at six atmospheres pressure

Refilling of the swimbladder at depth is slow; a small fraction of the original volume was replaced in 24 h and about half in 48 h. In shallow water the original volume would have been restored in 18–24 h (Fänge and Wittenberg, 1958; Wittenberg, 1958).

The partial pressure of argon in the secreted gas exceeded ambient in about one half of the fishes (Fig. 1A); and the partial pressure of nitrogen exceeded ambient in about one third of the individuals (Fig. 1B). The proportion of oxygen in all samples was 75% or greater.

Secretion of argon by fishes in air-equilibrated water

The enhancement of argon ranged from a small number to approximately 2. The enhancement tends to be small in the initial secreted sample and greater in subsequent samples (Fig. 2). The enhancement of argon is not different in fish maintained at 1 atm or 6 atm (Fig. 2).

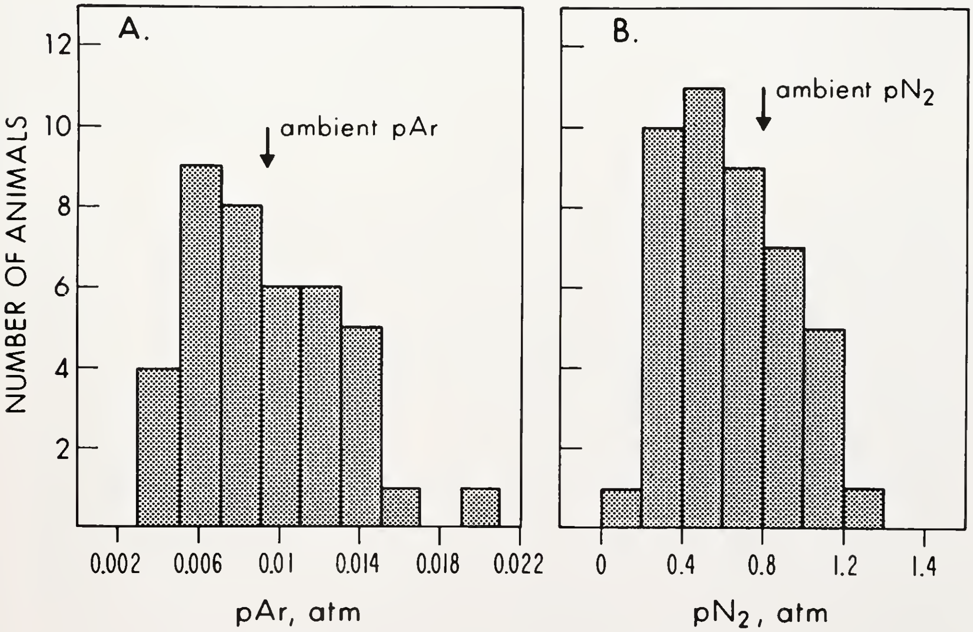

FIGURE 1. Partial pressure of argon, (pAr) A, and partial pressure of nitrogen, (pN_2) B, in gases brought into the experimentally emptied swimbladder of toadfish maintained at 50 m depth, equivalent to 6 atm total pressure. The arrows indicate the partial pressures of argon and nitrogen in air-equilibrated sea water.

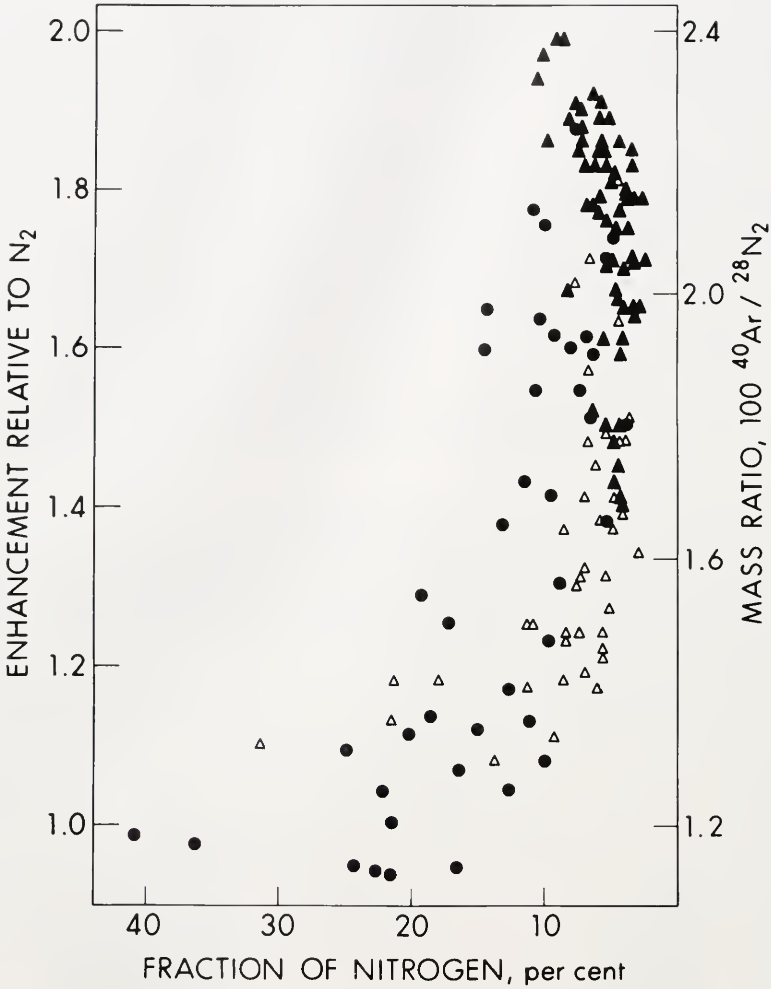

FIGURE 2. Enhancement of argon in gases brought into the experimentally emptied swimbladder at 50 m depth and at the surface. Enhancement, defined in the text, is: $(Ar/mass\ 28)_{secreted} \div (Ar/mass\ 28)_{ambient}$. Toadfish maintained at 50 m depth (dots). Toadfish maintained in shallow tanks (triangles); initial samples of replaced gas (open triangles); subsequent samples (closed triangles).

Simultaneous transport of several inert gases

With the exception of xenon, the enhancement of each inert gas tended to be small in initial samples and approach a larger, reproducible value in subsequent samples (Table I). The scatter of the data (standard deviation) tends to be less in the third and subsequent samples, than in the initial and second sample. The mean enhancements of the gases in the third, fourth, and fifth successive samples are compared in Table II with some properties of the gases.

In Figure 3 the enhancements of xenon, krypton, neon, and helium are plotted against the enhancement of argon in the same gas sample. These relations are linear with slopes characteristic of each gas.

TABLE I

Enhancement of noble gases brought into the swimbladder in successive replenishments of the swimbladder gas. Enhancement is defined in the text. Numbers are given as the mean and standard deviation. The number of samples is given in parentheses.

	Enhancement			
Sample	Neon	Argon	Krypton	Xenon
1	0.463 ± 0.08 (6)	1.36 ± 0.17 (15)	2.30 ± 0.45 (14)	3.95 ± 0.34 (5)
2	0.530 ± 0.12 (6)	1.72 ± 0.14 (15)	3.03 ± 0.38 (14)	4.45 ± 0.24 (7)
3	0.595 ± 0.05 (2)	1.87 ± 0.04 (9)	3.22 ± 0.11 (8)	4.27 ± 0.31 (6)
4	0.677 ± 0.07 (2)	1.92 ± 0.06 (8)	3.23 ± 0.15 (7)	4.15 ± 0.21 (4)
5		1.97 (1)	3.26 (1)	4.23 (1)
	mean of samples, 3, 4, and 5			
	0.636 ± 0.07 (4)	1.90 ± 0.05 (18)	3.23 ± 0.12 (16)	4.22 ± 0.26 (11)

DISCUSSION

In this study we use the shallow-living toadfish to explore a phenomenon of deep waters, the secretion of nitrogen and other inert gases against high pressure. We first establish that toadfish can bring inert gases into the swimbladder to greater than ambient partial pressure (Fig. 1A, 1B).

Wittenberg (1958) considered that the undoubted active accumulation of oxygen could provide the driving force for inert gas accumulation. He argued that oxygen might be secreted as a stream of small bubbles which would be invaded by the inward diffusion of nitrogen and other gases drawn from a shell of water surrounding each bubble, and that the oxygen bubbles would sweep their cargo of inert gases into the swimbladder. The mass of inert gas in each bubble would not exceed the mass contained in an unstirred layer of water around that bubble, and,

TABLE II

Enhancement of the noble gases and oxygen compared to their diffusivity and solubility in water. Relative diffusivity is approximated from the reciprocal of the square root of the molecular weight. The Bunsen solubility coefficient is the volume of gas (at 0°C, 1 atm pressure) absorbed per unit volume of water at the temperature of measurement when the partial pressure of that gas is 1 atm.

Gas	Molecular Weight	Diffusivity Relative to Nitrogen	Bunsen Solubility Coefficient	Solubility Relative to Nitrogen	Enhancement
Helium	4.00	2.65	0.00873	0.560	0.51
Neon	20.18	1.18	0.01045	0.670	0.636
Nitrogen	28.02	1.00	0.01559	1.00	1.00
Oxygen	16.00	0.94	0.03103	1.99	2.0
Argon	39.94	0.84	0.03412	2.19	1.90
Krypton	83.80	0.58	0.06264	4.02	3.23
Xenon	131.3	0.46	0.1122	7.20	4.22

Mean enhancement of noble gases from Table I. Enhancement of oxygen is from toadfish essentially lacking circulating erythrocytes (Wittenberg *et al.*, 1981). Enhancement of helium is from the second and third successive samples secreted by an eel (Wittenberg, 1958). Solubilities from Weiss (1970, 1971a, and 1971b), Benson and Krause (1976), Wilhelm *et al.* (1977) and Weiss and Kyser (1978), at 20°C.

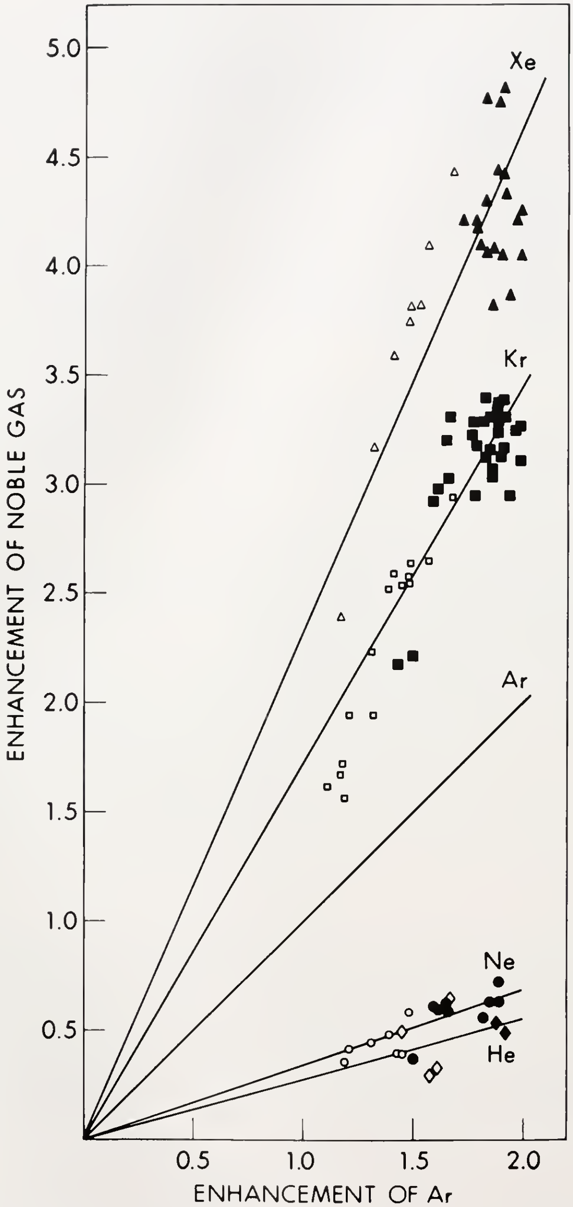

FIGURE 3. Enhancement of noble gases relative to the enhancement of argon. Enhancement is defined in the text. Points represent individual gas samples. A line of unit slope is drawn for argon. The other lines are least squares fits constrained to pass through the origin. Data for helium are from Wittenberg (1958). Open symbols, initial samples of secreted gas. Closed symbols, subsequent samples. Four successive gas samples were usually obtained from each fish.

since unstirred layers tend to be always of about the same thickness, would be a function of the size of the bubble independent of pressure. The mass of oxygen in each bubble would depend on the size of the bubble and the pressure. We reason that on this hypothesis, unless bubble size is dependent on depth, the fraction of nitrogen in the secreted gas should be much less at depth. The data presented in Figure 2 show no difference in gas composition with depth. We reject the hypothesis.

These findings remove all experimental support for the concept that oxygen secretion provides the driving force for nitrogen transport. They have no bearing, however, on the manner in which gases are released from the surface of the gas gland. It may well be that cytoplasmic crystaloid bodies (Copeland, 1969; Brooks, 1970; Morris and Albright, 1975, 1977) and surface specializations (Copeland *et al.*, 1980) serve as nucleation sites for gas release.

We turn now to the simultaneous transport of the several inert gases at one atmosphere total pressure. The measured enhancements in individual gas samples, for instance of argon in Figure 2, are scattered over an enormous range. The net accumulation of gas in the toadfish swimbladder represents an ongoing balance of secretion by the gas-secreting complex of the anterior swimbladder chamber and resorption by the specialized resorbing epithelium of the posterior chamber (Fänge and Wittenberg, 1958). Although the experimental protocol was designed to emphasize gas secretion and minimize resorption, individual differences in the extent of resorption will remain. Individual gas samples differed in the intensity or vigor of enhancement of all of the gases in that sample at the time the sample was elaborated. The changes which we see in intensity or vigor do not stem from variations in the rate of secretion, which remained always about the same, and vary only weakly with the fraction of nitrogen in the sample (Fig. 1).

The enhancement of each gas tends toward a high constant value if the first samples of gas secreted by each fish are put aside. The mean of the enhancements of the third, fourth, and fifth successive samples are ranked in Table II together with some properties of the gases. In the absence of blood hemoglobin to which it may bind, oxygen will behave as any other gas. The enhancement of oxygen in fish essentially lacking circulating hemoglobin (Wittenberg *et al.*, 1981) is ranked with the enhancements of the noble gases in Table II. The enhancements of the gases bear no simple relation to molecular weight or to its correlate, diffusivity.

The enhancement of each gas may be compared to that of others within any particular sample. This is done in Figure 3 where the enhancements of xenon, krypton, neon, and helium are plotted against the enhancement of argon in the selfsame sample. Linear relations emerge with slopes characteristic of each gas. These slopes express the relations:

$$\left(\frac{\text{noble gas}}{\text{argon}}\right)_{\text{secreted}} \div \left(\frac{\text{noble gas}}{\text{argon}}\right)_{\text{ambient}}$$

These are the smoothed enhancements, normalized relative to argon. These, together with values for nitrogen and oxygen taken from Tables I and II, are used to construct Figure 4.

Many solution properties of the gases may correlate with enhancement; since these properties are intimately interrelated they will change together in a homologous series of gases. We focus attention on solubility solely because, in salting out, we perceive a mechanism by which gas solubility may be depressed and partial pressure elevated. A plot, Figure 4, of the enhancements relative to argon versus the solubilities of the gases in water shows a linear function which falls off at higher values. Helium, neon, nitrogen, argon, and oxygen follow the linear relation fairly closely; krypton deviates somewhat, and xenon deviates substantially. We conclude that enhancement is closely related to solubility.

Salting out of gases from aqueous solution obeys the empirical Setschenow

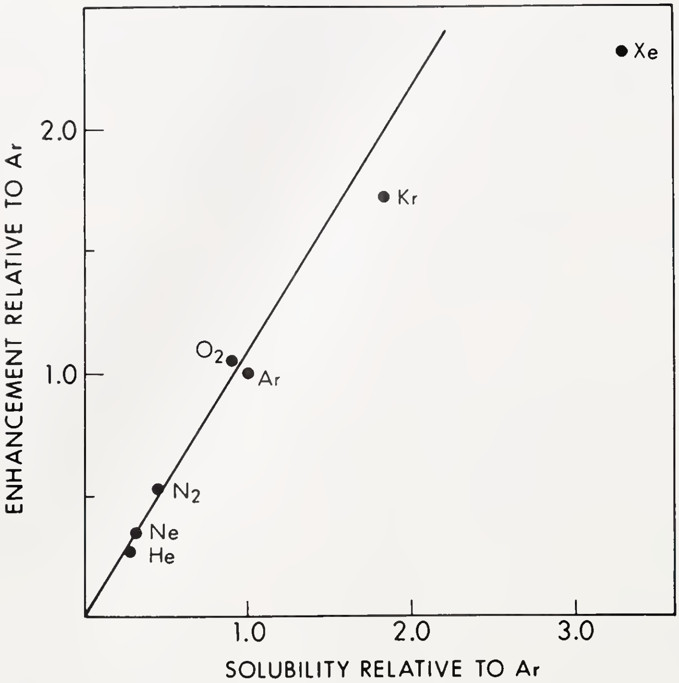

FIGURE 4. Enhancement relative to argon of noble gases, nitrogen and oxygen as a function of their solubility in water. Enhancement is defined in the text. Enhancements of the noble gases are the slopes of the lines in Figure 3. Enhancements of nitrogen and oxygen estimated from the data of Table I taking the enhancement of argon relative to nitrogen as 1.90.

relation which states that at constant temperature the logarithm of the solubility is a linear function of the salt concentration:

$$\log (S_O/S) = kC \qquad (1)$$

here S_O and S are bunsen solubility coefficients in the absence and presence of salt; k is a constant different for each gas and each solute, and C is the concentration of the solute. All of the gases considered here follow this relation closely without systematic deviation (Morrison and Billett, 1952; Morrison and Johnstone, 1955; Weiss, 1970, 1971a, 1971b; Weiss and Kyser, 1978). Values of the salting out coefficient are collected in Table III. We note that the salting out coefficients of the several gases are of similar magnitude. From this and from equation 1, we note that the ratio S_O/S, for a given increment of solute concentration, is similar for all gases studied. Thus, a given increment of added solute drives off the same fraction of each dissolved gas. It follows that the composition of the gases salted out is similar to the composition of the gases dissolved in the plasma. The amount of each gas driven off by any increment of solute is proportional to its solubility and the partial pressure (of that gas) with which the plasma was equilibrated at the gill. The more soluble gases will be enriched in the gases brought into the swimbladder.

The "single concentrating effect" expresses the equilibrium increase in the partial pressure of each gas brought about by salting out. Counter-current multiplication of the single concentrating effect may be looked on as a kinetic process in which transport of the more diffusible gases is favored since they pass more

TABLE III

Coefficients for salting out of some gases by sodium chloride.

Gas	[1]Data of Weiss (1971a, 1971b) and of Weiss and Kyser (1978) 20°C	[2]Data of Morrison and Johnstone (1955) and Morrison and Billet (1952) 25°C	[2]Data of Eucken and Herzberg (1950) 20°C
Nitrogen	0.136	0.121	
Oxygen	0.127	—	
Helium	0.096	0.081	
Neon	0.106	0.097	
Argon	0.128	0.133	0.139
Krypton	0.134	0.146	
Xenon	—	0.149	0.150

[1] Data for salinity of sea water has been recalculated for equivalent ionic strength of sodium chloride, taking S = 10 0/00 equivalent to I = 0.20239.

[2] Expressed as moles per 1000g water.

rapidly from outflowing to inflowing capillaries of the rete mirabile (Kuhn *et al.*, 1963). This kinetic effect would tend to decrease the enhancements of the relatively less diffusible krypton and xenon and cause them to depart from the linear relation shown in Figure 4. In fact, the measured resistance of the walls of the retial capillaries to gas diffusion is large (Rasio and Goreski, 1979).

The "single concentrating effect" (Kuhn *et al.*, 1963) that can be generated by salting out is small; the addition of even 0.02 *M* salt would depress solubility less than 1%; and the rate of inert gas secretion estimated on this basis is much less than the measured rate (Scholander, 1954; Kuhn *et al.*, 1963; Enns *et al.*, 1967). For this reason we consider the potentially greater effect that could be generated by perturbing the binding of gases to blood proteins. Plasma proteins do not bind nitrogen (Van Slyke *et al.*, 1934; Steen 1963). Human hemoglobin binds significant amounts of nitrogen (Van Slyke *et al.*, 1934), oxygen at sites other than the heme (Sendroy *et al.*, 1934) and, at known sites, xenon (Schoenborn, 1965). Binding of nitrogen, oxygen and argon in the erythrocytes of some fishes is depressed about 5 per cent by acidification, with apparent pK = 7.5 (Steen, 1963; Abernethy, 1972). However we show that erythrocytes play no necessary part in nitrogen secretion (Wittenberg *et al.*, 1981). We conclude that non-specific gas binding to proteins plays at most a minor role in inert gas secretion.

All facts presented here support the hypothesis that salting out of inert gases from their solution in the blood plasma generates the small "single concentrating effect." High pressures of these gases may be generated by counter-current multiplication of the single concentrating effect in the rete mirabile. Substances which may be responsible for salting out the inert gases are lactate anion, carbon dioxide and bicarbonate anion (Wittenberg *et al.*, 1981).

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LITERATURE CITED

Abernethy, J. D. 1972. The mechanism of secretion of inert gases into the fish swimbladder. *Aust. J. Exp. Biol. Med. Sci.* **50:** 365–374.

Benson, B. B., and D. Krause. 1976. Empirical laws for dilute aqueous solutions of non-polar gases. *J. Chem. Phys.* **64:** 689–709.

Brooks, R. E. 1970. Ultrastructure of the physostomatous swimbladder of rainbow trout (*Salmo gairdneri*). *Z. Zellforsch.* **106:** 473–483.

Copeland, D. E. 1969. Fine structural study of gas secretion in the physoclistous swimbladder of *Fundulus heteroclitus* and *Gadus callarius* and in the euphysoclistous swimbladder of *Opsanus tau*. *Z. Zellforsch.* **93:** 305–331.

Copeland, D. E., R. L. Haedrich, and J. B. Wittenberg. 1980. Gas secretion in the swimbladder of shallow water fishes compared to a deep ocean fish (3000 meters). *Biol. Bull.* **159:** 449.

Douglas, E. L. 1967. Studies of gas secretion in the swimbladder of fishes. Ph.D. Dissertation, University of California, San Diego. Pp. 117 University Microfilms Inc., Ann Arbor, Michigan.

Enns, T., E. L. Douglas, and P. F. Scholander. 1967. Role of the swimbladder rete of fish in secretion of inert gas and oxygen. In: *Advances Biol. Med. Phys.* (J. H. Lawrence, and J. W. Cofman, Eds.), Vol. **2,** Pp. 231–244, Academic Press, New York.

Eucken, A., and G. Herzberg. 1950. The Salting-out effect and ion hydration. *Z. Physikal. Chem.* **195:** 1–23.

Fänge, R., and J. B. Wittenberg. 1958. The swimbladder of the toadfish (*Opsanus tau*). *Biol. Bull.* **115:** 172–179.

Haldane, J. S. 1898. Secretion and absorption of gas in the swimming-bladder and lungs. Part 1—Swimming-bladder. *Sc. Progr.* **7:** 120–130.

Hüfner, G. 1892. Zur physikalischen Chemie der Schwimmblasengase, *Arch. Anat. u. Physiol., Physiol. Abt.* 54–80.

Koch, H. 1934. L'emission de gas dans la vesicule gazeuze des poissons. *Rev. Questions Scientifique,* series 4, **26:** 385–409.

Kuhn, W., and H. J. Kuhn. 1961. Multiplikation von Aussalz-und anderen Einzeleffekten fur die Bereitung hoher Gasdrucke in der Schwimmblase. *Z. Elektrochem.* **65:** 426–439.

Kuhn, W., A. Ramel, H. J. Kuhn, and E. Marti. 1963. The filling mechanism of the swimbladder. Generation of high gas pressures through hairpin countercurrent multiplication. *Experientia.* **19:** 497–511.

Morris, S. M., and J. T. Albright. 1975. The ultrastructure of the swimbladder of the toadfish, *Opsanus tau* L. *Cell Tissue Res.* **164:** 85–104.

Morris, S. M., and J. T. Albright. 1977. Cytochemical study of the lamellar bodies in the swimbladder of the toadfish *Opsanus tau* L. *Cell and Tissue Res.* **185:** 77–87.

Morrison, T. J., and F. Billett. 1952. The salting out of non-electrolytes, Part II. The effect of variation of non-electrolyte. *J. Chem. Soc.* Pp. 3819–3822.

Morrison, T. J., and N. B. B. Johnstone. 1955. The salting out of non-electrolytes. Part III. The inert gases and sulfur hexafluoride. *J. Chem. Soc.* Pp. 3655–3659.

Powers, E. B. 1932. The relation of the respiration of fishes to environment. *Ecol. Monographs* **2:** 385–473.

Rasio, E. A., and C. A. Goreski. 1979. Capillary limitation to oxygen distribution in the isolated rete mirabile of the eel (*Anguilla anguilla*). *Circ. Res.* **44:** 498–503.

Schloesing, T., and J. Richard. 1896. Recherche de l'argon dans les gaz de la vessie natatoire des poissons et des Physalies. *Compt. Rend. Acad. Sci.* (Paris) **122:** 615–617.

Schoenborn, B. P. 1965. Binding of xenon to horse haemoglobin. *Nature* **208:** 760–762.

Scholander, P. F. 1954. Secretion of gases against high pressures in the swimbladder of deep sea fishes. II. The rete mirabile. *Biol. Bull.* **107:** 260–277.

Scholander, P. F., L. van Dam, and T. Enns. 1956. Nitrogen secretion in the swimbladder of whitefish. *Science* **123:** 59–60.

Sendroy, J., R. T. Dillon, and D. D. Van Slyke. 1934. Studies of gas and electrolyte equilibria in blood. XIX. The solubility and physical state of uncombined oxygen in blood. *J. Biol. Chem.* **105:** 597–632.

Steen, J. B. 1963. The physiology of the swimbladder of the eel *Anguilla vulgaris*. I. The solubility of gases and the buffer capacity of the blood. *Acta Physiol. Scand.* **58:** 124–137.

SUNDNES, G., T. ENNS, AND P. F. SCHOLANDER. 1958. Gas secretion in fishes lacking rete mirabile. *J. Exp. Biol.* **35:** 671–676.

TAIT, J. S. 1956. Nitrogen and argon in salmonoid swimbladders. *Canad. J. Zool.* **34:** 58–62.

VAN SLYKE, D. D., R. T. DILLON, AND R. MARGARIA. 1934. Studies of gas and electrolyte equilibria in blood. XVIII. Solubility and physical state of atmospheric nitrogen in blood cells and plasma. *J. Biol. Chem.* **105:** 571–596.

WEISS, R. F. 1970. The solubility of nitrogen, oxygen and argon in water and sea water. *Deep-Sea Res.* **17:** 721–735.

WEISS, R. F. 1971a. The effect of salinity on the solubility of argon in sea water. *Deep Sea Res.* **18:** 225–230.

WEISS, R. F. 1971b. Solubility of helium and neon in water and seawater. *J. Chem. Eng. Data.* **16:** 235–241.

WEISS, R. F., AND T. K. KYSER. 1978. Solubility of krypton in water and seawater. *J. Chem. Eng. Data.* **23:** 69–72.

WILHELM, E., R. BATTINO, AND R. J. WILCOCK. 1977. Low pressure solubility of gases in liquid water. *Chem. Rev.* **77:** 219–262.

WITTENBERG, J. B. 1958. The secretion of inert gas into the swimbladder of fish. *J. Gen. Physiol.* **41:** 783–804.

WITTENBERG, J. B., M. J. SCHWEND, AND B. A. WITTENBERG. 1964. The secretion of oxygen into the swimbladder of fish. III. The role of carbon dioxide. *J. Gen. Physiol.* **48:** 337–355.

WITTENBERG, W., D. K. WITTENBERG, AND J. B. WITTENBERG. 1981. Secretion of nitrogen into the swimbladder of fish. I. Secretion by fishes nearly lacking circulating hemoglobin. Role of the rete mirabile. *Biol. Bull.* **161:** 426–439.

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